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<th>Cell proliferation and expression of Cbfa-1 in a peripheral osteo-chondroma arising from the mandibular oral mucosa of an edentulous alveolar ridge</th>
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INTRODUCTION

Osteo-chondromas around the oral area have been reported on the mandibular condyle, the mandibular angle, and the coronoid process. However, osteo-chondroma tumors arising from the periphery of the oral mucosa of the alveolar ridge are extremely rare. The mandibular bone (except for the mandibular condyle) normally grows and is...
remodeled by intramembranous ossification without endochondral ossification. Osteochondromas or cartilaginous mesenchymomas in the field of orthopedics have mainly been reported in the epiphysis of long bones. This suggests that chondromas can normally be produced where cartilage is naturally present. In contrast, reactive bone or cartilage formation is known to involve calcareous degeneration, i.e. hypercalcemia or dystrophic calcification. It is important to be able to distinguish hard tissue produced by cells from that resulting from degeneration in order to distinguish the characteristics of the neoplasm. The purpose of this paper is to report a rare case of osteo-chondroma which arose from the edentulous alveolar ridge and to discuss the mechanism of cartilage formation in the tumor.

**CASE REPORT**

A 72-year-old female was admitted to a private dental office on September of 2001, with the complaint of a poor fit of her edentulous denture, which was associated with a painless swelling. This swelling had appeared one month earlier on her oral mucosa in the middle region of the mandibular alveolar ridge. The lesion was covered with normal mucosa without any apparent abnormality. She had no history of trauma in that region other than the tooth extraction many years earlier. Following the diagnosis of a denture fibroma, the tumor was resected on October 16th, 2001. The resected material was immediately fixed with 10% formalin and sent to the Department of Clinical Pathophysiology, Tokyo Dental College, for pathological diagnosis.

**PREPARATION OF TISSUE**

After the specimen was rinsed with water, it was dehydrated with a graded series of ethanol before being embedded in paraffin. Paraffin sections about 5μm in thickness were cut and stained with hematoxylin-eosin or PAS-alcian blue. Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) and for core binding factor a-1 (Cbfa-1) was also performed.

For immunohistochemical staining, the PCNA monoclonal antibody (diluted at 1:100, PC-10, PCNA, DAKO, Denmark) and the Cbfa-1 monoclonal antibody (diluted at 1:1,500, supplied by Dr. Sasaguri, Department of Orthodontics, Kanagawa Dental College, Japan) were used. Paraffin sections were deparaffinized with xylene and washed with 100% alcohol and distilled water. Endogenous peroxidase activity was blocked by incubating the sections with 3% H₂O₂ in methanol for 30 min. To prevent non-specific reactions, sections were incubated with 10% serum for 30 min in a 100% humidity chamber. After washing in PBS, the sections were incubated with the secondary antibody for 30 min in a humidity chamber. After further washing in PBS three times for 5 min each, the sections were stained with 3,3′-diaminobenzidine for 5 min and were finally counterstained with hematoxylin.

**PATHOLOGICAL FINDINGS**

The lesion was round, and its size was 0.9×0.4×0.3 cm (Fig. 1). The mass consisted mainly of mesenchymal cells without any kind of atypia and was intact to the stratified squamous epithelium of the oral mucosa. Fibrous bone surrounded by osteoblasts was seen in the deep areas, and chondrocytes with cartilage stroma stained by PAS-alcian blue were also observed (Figs. 2, 3).

Almost all of the mesenchymal cells around the bone and the cartilage tissues reacted with the PCNA monoclonal antibody (Fig. 4), but only the mesenchymal cells around the bone and the cartilage tissue were positive for Cbfa-1 (Fig. 5).

**DISCUSSION**

Cartilaginous tissues arising on edentulous
A CASE OF PERIPHERAL OSTEO-CHONDROMA

Fig. 1 Hematoxylin-eosin staining in loupe findings. Mesenchymal cells proliferate in the mass without atypia. (×20)

Fig. 2 Hematoxylin-eosin staining at high magnification. Fibrous bone and cartilage tissue are observed. (×200)

Fig. 3 PAS-alcian blue staining. Cartilage stroma is stained. (×200)

Fig. 4 Immunohistochemical staining with PCNA. Many mesenchymal cells are strongly positive around hard tissue. (×200)

Fig. 5 Immunohistochemical staining with Cbfa-1. Many mesenchymal cells are positive, but only around the hard tissue. (×200)
oral mucosa of the alveolar ridge are extremely rare, because cartilaginous tissue is present only at the head of the mandibular condyle in the oral area. This explains why there are many case reports of chondromas or osteo-chondromas arising from the mandibular condyle\(^2,3,9,15,16,21\). Additionally, there are some reports of osteo-chondromas arising from the mandibular angle\(^14\), and the tongue\(^18\). Therefore, cartilaginous tumor tissues appear in four types of location; (1) an area where cartilaginous tissues are present like the mandibular condyle, (2) around bone tissues like the mandibular angle, (3) in soft tissues like the tongue, and (4) in calcareous degenerations like dystrophic calcification. This case, which arose from the alveolar ridge, is of the second type (around bone tissue).

The PCNA antibody has been used in many kinds of studies to evaluate tumor cell activity or wound healing, since Bravo \textit{et al.}\(^4\) first reported it in 1987\(^12,17\). Many PCNA positive mesenchymal cells were observed around the bone and cartilage in this lesion, indicating that the hard tissue was manufactured by cells and was not due to dystrophic calcification. Cbfa-1 is a transcription factor that belongs to the runt domain gene family\(^5–7\) and is a regulatory factor not only in osteoblast maturation, but also in chondroblast maturation\(^5,7\). Furthermore, Cbfa-1 regulates not only expression of bone and cartilage-related genes. Positive staining indicates that the calcification is not dystrophic. Although it is often difficult to distinguish between preosteoblasts and fibroblasts, immunohistochemical staining with the Cbfa-1 antibody can specifically identify osteogenic cells such as osteoblasts or chondroblasts. Bone formation by osteoblasts can occur normally around bone tissue, but it also occurs in areas of calcareous degeneration, such as hypercalcemia or dystrophic calcification; the latter process is usually termed reactive. This fact suggests that osteogenic neoplasms need to be distinguished from reactive bone formation. Furthermore, in this case, the PCNA positive cells surrounded the Cbfa-1 positive ones. These high activity cells were revealed by the stain to be osteogenic. The differential diagnosis between a tumor and a reactive type of a lesion of hard tissue is decided by determining whether or not osteoblasts surround the bone tissue\(^20\). Further, osteogenic stem cells are known to arise and initiate hard tissue formation in response to environmental factors\(^1,8\). Recently, the receptor activator of the nuclear factor ligand and osteoprototegen (RANKL/OPG) system, a novel cytokine system of cellular receptors, has been studied with regard to bone cell biology, including consideration of bone disease and skeletal complications in rheumatoid arthritis\(^10,11,19\). In particular, Hofbauer \textit{et al.} reported that the RANKL system includes important regulators of calcification and has a crucial role in extraskeletal calcium handling\(^20\). There is some possibility that the lesion reported here is related to the RANKL/OPG system.

Based upon our results, we suggest that a few mesenchymal cells which originated from the peripheral periosteum or the bone marrow arose in response to the tooth extraction, and that they thereafter proliferated and differentiated by degrees. Therefore, the cartilage tissue was not produced by dystrophic calcification. Instead, the cells in this mass produced cartilage in part because they were surrounded by many cells which reacted immunohistochemically with Cbfa-1.

Our results with this case indicate that immunohistochemical staining for PCNA and for Cbfa-1 can be used to investigate lesions with bone or cartilage formation and to distinguish those produced by osteogenic cells from those that are just reactive and produced by dystrophic calcification.

\textbf{REFERENCES}


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