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Osteofibrous Dysplasia-Like Adamantinoma
- A Case Report with its Immunohistochemical and Ultrastructural Studies -

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Osteofibrous dysplasia (OFD)-like adamantinoma is a rare skeletal tumor that is characterized by the predominant OFD-like pattern with scattered epithelial nests. Adamantinoma shares clinical features (the majority of lesions in the tibia and the prevalent age group), radiologic findings (radiolucency with sclerotic shadow), and pathologic similarities (particularly the presence of scattered cytokeratin-positive stromal cells) with OFD. We describe a case of OFD-like adamantinoma. Epithelial cell nests express the epithelial membrane antigen, pancytokeratin, CK14, and collagen type IV. Ultrastructurally, the oval to spindle cells in the epithelial foci had abundant tonofilaments, and well-formed desmosomes with dense plaques, of which well preserved desmosomes are demonstrated for the first time in OFD-like adamantinoma. These immunohistochemical and ultrastructural findings further support that the origin of epithelial cells of classic and OFD-like adamantinoma are epithelial cells transformed from fibroblastic cells in the proliferating osteofibrous tissue.

Key Words : Adamantinoma-Ossifying Fibroma-Microscopy, Electron-Immunohistochemistry

Osteofibrous dysplasia (OFD)-like adamantinoma is a histological subtype of adamantinoma. It has been considered as a part of the morphologic spectrum of adamantinoma. OFD-like adamantinoma is also referred as differentiated or regressing adamantinoma.[1] It differs from classic adamantinoma: it has predominant proportion of OFD-like pattern and its prognosis after conservative treatments is better than the classic adamantinomas in long bone.[2]

Presently, only one case of adamantinoma of the tibia with predominant features of fibrous dysplasia has been described in Korea.[3] Here, we report a case of OFD-like adamantinoma and briefly review the literature.

CASE REPORT

The patient was a 21-year-old Korean woman suffering from a palpable mass in the left lower leg, which was accompanied by exertional pain for 8 years. The patient as well as her family members did not show the cafe-au-lait spots. The anteroposterior and lateral views of the left tibia showed a well-defined intracortical radiolucent lesion with sclerotic margin at the diaphysis (Fig. 1). The radiological differential diagnosis was more like fibrous dysplasia and OFD than adamantinoma. The serum level of alkaline phosphatase was normal. The patient was treated by the en bloc resection without adjuvant treatment. 12 months later, the fracture at the operation site in the tibia occurred, and a cast was done. She was regularly monitored by bone scans and X-rays for 20 months after the operation. During the period, metastasis or recurrence was not detected.

PATHOLOGICAL FINDINGS

The resected specimen was fixed in 10% buffered formalin

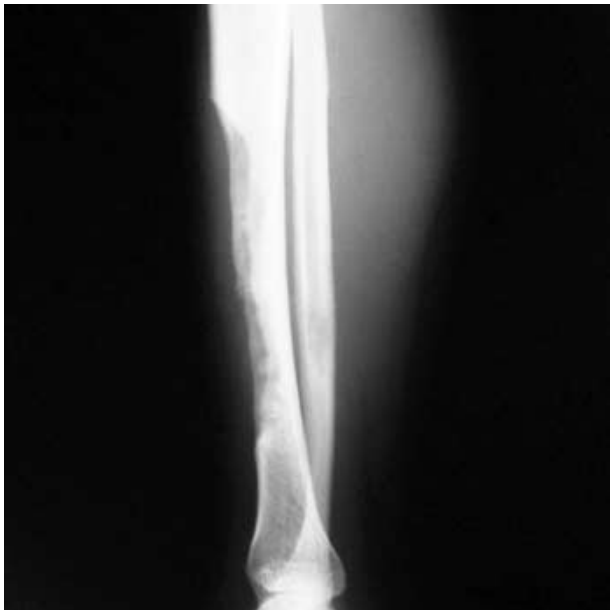

Fig. 1. Plain roentgentogram of the lateral view of the tibia shows multifocal osteosclerotic lesions intermixed with lytic lesions at the intracortical portion of the shaft.

solution. After decalcification processing in 10% nitric acid, the specimen was embedded in paraffin and stained with hematoxylin and eosin. Immunohistochemistry was performed by using the avidin-biotin peroxidase complex method. The following antibodies were used: pancytokeratin (AE1/AE3; Zymed, San Francisco, CA, USA; 1:50), cytokeratin 8 (CK8; Dako, Glostrup, Denmark; 1:80), cytokeratin 10 (CK10; Neomarkers, Fremont, CA, USA; prediluted), cytokeratin 14 (CK14; Neomarkers; prediluted), cytokeratin 19 (CK19; Dako; 1:80), epithelial membrane antigen (E29; DAKO; 1:50), smooth muscle actin (1A4; Zymed; 1:100), S-100 protein (polyclonal, Dako; 1:1200), vimentin (V9; Zymed; 1:100), factor VIII-related antigen (Z002; Biogenesis, Poole, UK; 1:150), CD34 (QBEnd 10; Dako; 1:100), collagen type IV (Dako; 1:50), laminin (Biogenesis; 1:100), TGF-beta 1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:50), fibronectin (Biogenesis; 1:100), p53 protein (Zymed; 1:50), p16 protein (Santa Cruz Biotechnology; 1:50) and Ki-67 (Zymed; 1:100). For the electronmicroscopic examination, paraffin block was deparaffinized and fixed in 2.5% glutaraldehyde, treated with 1% osmium with propylene dioxide, embedded in Epok 812 (Oken Shoji Ltd., Tokyo, Japan), and sectioned. The thin sections (1 μm) were stained with toluidine blue and Azure B solutions to ascertain that the desired cells were in the block. Subsequently, the sample was examined with a transmission scanning electron microscope (H-7100, Hitachi High-Technologies Corporation, Tokyo, Japan) at the accelerating voltage of 75 kv.

Table 1. Immunohistochemical and ultrastructural summary of the present case

	Epithelial component	Fibro-osseous component
Immunohistochemical findings		
Extracellular matrix materials		
Laminin	–	–
Collagen type IV	Focal +	–
Fibronectin	–	–
Pancytokeratin (AE1/AE3)	+	Scattered +
Cytokeratin 8	–	–
Cytokeratin 10	–	–
Cytokeratin 14	+	–
Cytokeratin 19	–	–
TGF-beta 1	+	+
Epithelial membrane antigen	Focal +	–
Vimentin	–	+
S-100 protein	–	–
Smooth muscle actin	–	Focal +
CD34	–	–
Factor VIII-related antigen	–	–
p53 protein	–	–
p16 protein	–	–
Ki-67 labeling index	0%	0%
Ultrastructural findings		
Morphology of tumor cells	Spindle-shaped cells Oval-shaped cells	Spindle-shaped cells Oval cells
Well-formed desmosomes	+	–
Tonofilaments	+	–
Dense plaques	+	–
External lamina	–	–
Lipid vacuoles	–	+ (Oval shaped foam cells)
RERs	+	+ (Abundant in spindle shaped fibroblasts)

RER, rough endoplasmic reticulum.

Grossly measured, the tibia bone was 4.0 × 2.3 × 2.0 cm. On serial sectioning, the ill-defined yellowish soft lesion, measuring about 2.0 × 1.0 cm was identified just beneath the thickened periosteal cortical bone. Microscopically, the majority of the lesion was fibro-osseous tissue with foamy macrophages and loose fascicles of elongated fibroblast-like cells, often showing the storiform pattern in the fibrous stroma (Fig. 2A-C). The center of the lesion was irregular woven bone trabeculae. The periphery of the lesion was matured, fine, lamellar bones with prominent osteoblasts. The histologic findings were compatible with OFD. When carefully examined the entire specimen, however, squamous multifocal scattered small epithelial cell nests were observed (Fig. 2D, left). Thus, the case was

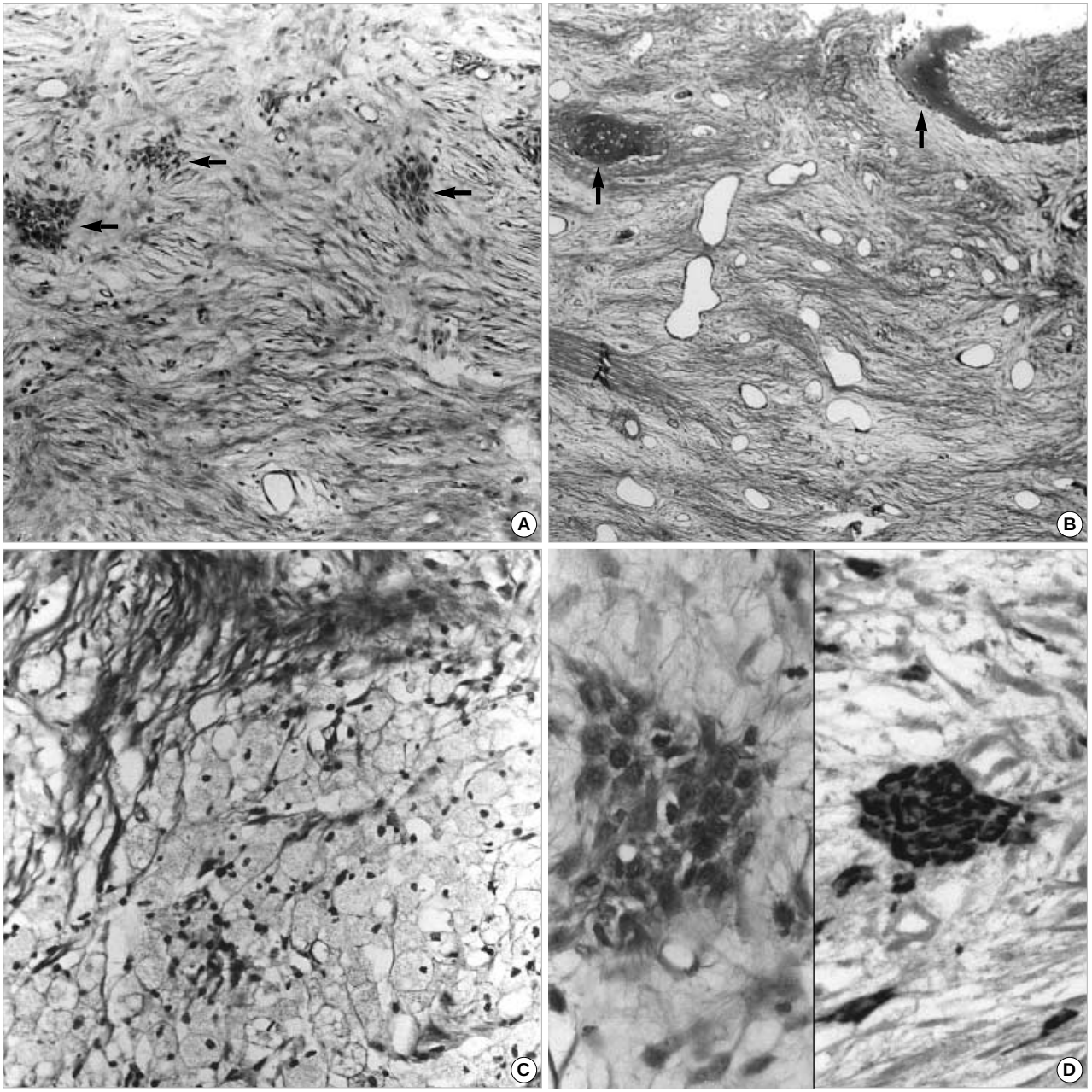

Fig. 2. (A) Fibrocollagenous lesion comprises spindle-shaped stromal cells forming fascicles and abundant vasculatures. Note several epithelial nests (arrows). (B) The predominant portion of osteofibrous dysplasia shows trabeculae of woven bone with osteoblastic rimming (arrows). (C) Many foam cells in the fibrous stroma are found. (D) High power view shows squamoid nests (left), and immunohistochemistry for pancytokeratin stains the cytoplasms of epithelial nests and a few scattered stromal spindle cells (right, pancytokeratin immunostain).

diagnosed as OFD-like adamantinoma. Immunohistochemically, the epithelial cell nests expressed the pancytokeratin (Fig. 2D, right), CK14 and epithelial membrane antigen. The nests were negative for CK10, CK19, CK8, p53 protein and p16 protein. Fibroblast-like spindle cells expressed vimentin and smooth muscle actin in some area. Some of the spindle-shaped cells in the fibro-osseous portion expressed CK14. Epithelial cell nests expressed collagen type IV in some area but not laminin. TGF-beta 1 was detected on fibroblasts, osteoblasts, and epithelial cells. Spindle cells in the fibrous area expressed fibronectin. Ultrathin section was performed on the selected portion of paraffin blocks containing squamous epithelial cell nests. The majority of the lesion of fibrocollagenous area contained spindle-shaped fibroblasts with abundant rough

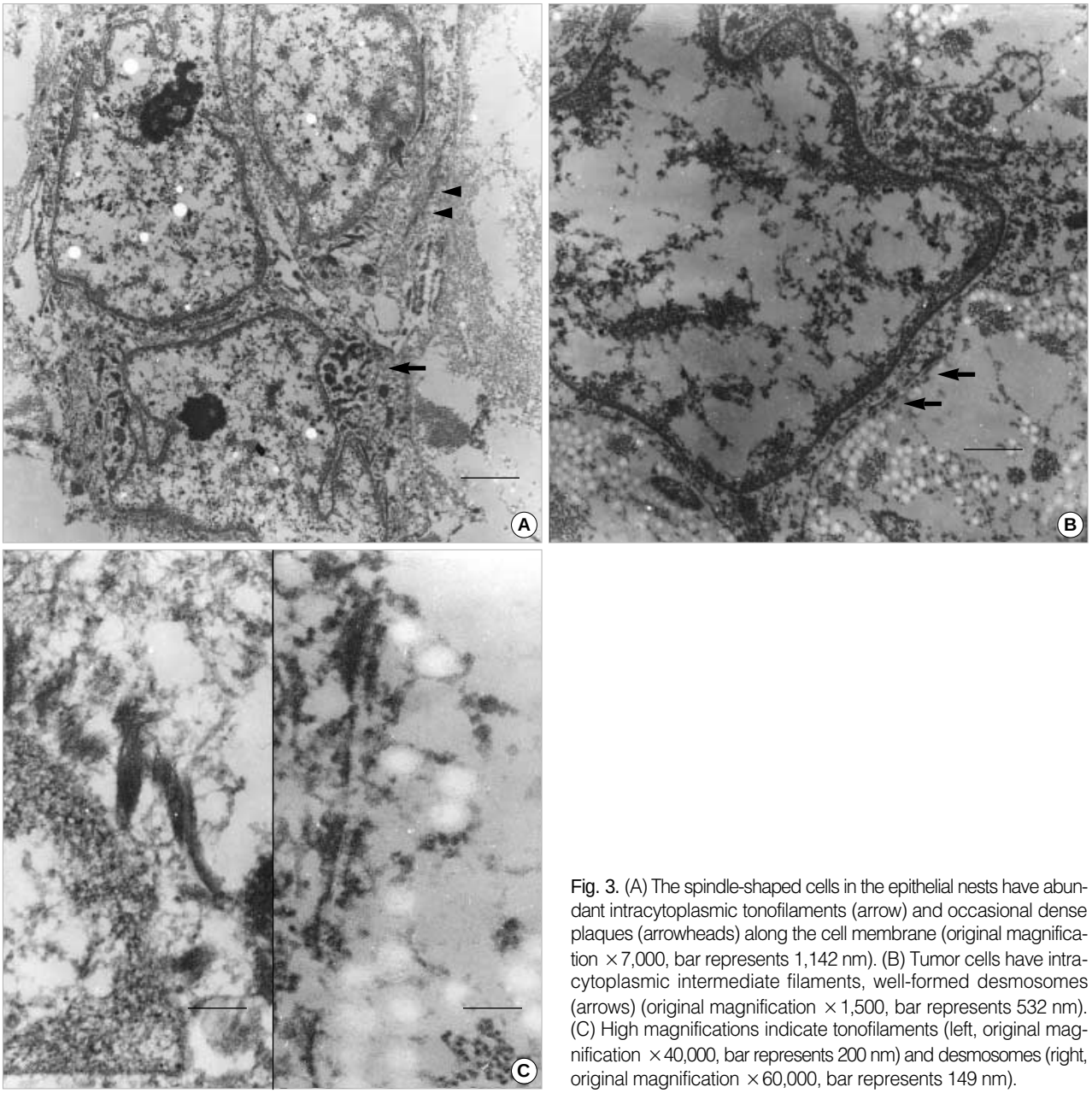

Fig. 3. (A) The spindle-shaped cells in the epithelial nests have abundant intracytoplasmic tonofilaments (arrow) and occasional dense plaques (arrowheads) along the cell membrane (original magnification ×7,000, bar represents 1,142 nm). (B) Tumor cells have intracytoplasmic intermediate filaments, well-formed desmosomes (arrows) (original magnification ×1,500, bar represents 532 nm). (C) High magnifications indicate tonofilaments (left, original magnification ×40,000, bar represents 200 nm) and desmosomes (right, original magnification ×60,000, bar represents 149 nm).

endoplasmic reticulums and oval macrophages with abundant fat vacuoles. The cytoplasms of oval cells as well as spindle shaped cells in the epithelial cell nests were filled with tonofilaments, intermediate filaments, dense plaques, and well-formed desmosomes along the cell membrane (Fig. 3). Neither spindle cells nor oval shaped cells showed external lamina around them. Collagen fibrils were present at the intercellular spaces. Table 1 shows the immunohistochemical and ultrastructural results of the present case.

DISCUSSION

The relationships between OFD-like adamantinomas and OFD are controversial.[1,4] Both are the osteolytic lesion in the cortex of the tibia in children that express the pancytokeratin in the scattered manner. In OFD without epithelial cells, the area expressing pancytokeratin varies from 2.5% to 93%.[5,6] OFD-like adamantinoma and OFD share various features. Table 2, however, shows their distinct features. The most reliable feature that distinguishes OFD-like adamantinoma from OFD is the presence of epithelial cell nests detected by light mi-

Table 2. Summary of distinguishing points between OFD-like adamantinoma and OFD

	OFD-like adamantinoma	OFD
X-ray		
Site	Anterolateral cortex of the tibia and fibula Diaphysis	Anterolateral cortex of the tibia and fibula Diaphysis
Characteristics	Intracortical radiolucency with sclerotic foci	Intracortical radiolucency with sclerotic foci
Pathology		
Fibro-osseous portion	+	+
Stromal spindle cells	CK: + (occasional)	CK: + (occasional)
Presence of epithelial nests	+	–
Epithelial nests	CK: +, vimentin: +/–	*
Ultrastructural findings	Dense plaque: + Desmosome: + Tonofilaments: + Basal lamina: +/–	Dense plaque: + Desmosome: – Tonofilaments: – Basal lamina: +/–

OFD, osteofibrous dysplasia; CK, cytokeratin; *Epithelial nests are absent in osteofibrous dysplasia.

croscopy. OFD-like adamantinoma rarely progresses to classic adamantinoma or metastasizes. It has been suggested that this unique variant is a part of the spectrum of lesions from classic adamantinoma at one end to OFD at the other end because immunohistochemical studies revealed that epithelial cells of both OFD-like adamantinoma and classic adamantinoma express the similar patterns of keratin[4,7,8]; CK5 and CK14 are expressed specifically by basal epithelial cells; CK14 is expressed by almost all cases of adamantinomas with or without OFD-like pattern. CK19 is expressed by the epithelial cell nests that are negative for CK4, CK10, CK8 and CK18. CK17, CK13 or CK7 occasionally stains the epithelial components of the adamantinoma. The profile of the expression of cytokeratin and TGF-beta may support the basal-epithelial-like differentiation in adamantinomas.[7,9] Other characteristic of the epithelial cell nests is the co-expression of cytokeratin and vimentin. The epithelial cell nests of the present case, however, did not express vimentin. Such case has been reported rarely.[3]

The origin of adamantinoma, classic as well as the OFD-like variant, remains controversial. Vascular, synovial and epithelial cells were initially proposed, but extensive immunochemical studies rule out the possibility.[10] Regarding the origin of epithelial components of OFD-like adamantinoma, two hypotheses are the most widely accepted. One is that resting ectopic embryonic cell is the origin of the tumor and the osteofibrous tissue is simply associated with tumor cells. The other is the clonal proliferating osteofibrous component transformed to mesenchymal-epithelial cells, in analogy to the development of epithelial formation in synovial sarcoma. The latter is supported by the fact that epithelial cells expressing FGF-2, EGF, and EGFR, proliferate vigorously may substantiate that epithelial cells stimulate the growth of fibroblast and epithelial cells via both autocrine and paracrine pathways.[11] The extracellular matrix component collagens type I and III, and fibronectin were generally detected in the fibrous tissue of adamantinoma. The epithelial cell nests did not express such extracellular matrix components. The close association of the epithelial cells and fibroblasts with the basement membrane proteins, collagen IV and laminin, are unique[12]; prominent areas with cohesive epithelial cells were surrounded by the continuous basement membrane, whereas less distinct epithelial islands were surrounded by the discontinuous basement membrane or no basement membrane at all. OFD-like adamantinomas expressed local punctate density or pericellular staining of basement membrane factors in fields of isolated keratin-positive cells. This distribution pattern fits well to the model of the mesenchymal-epithelial conversion, as observed in synovial sarcoma.[13] Only one ultrastructural study has been reported. The study showed the presence of tonofilaments in the fibroblasts, which is considered as the earliest step in the transformation of fibroblast to epithelial cells.[2] Although there has yet been no description about desmosomes or primitive cell junctions, well-formed desmosomes as well as tonofilaments in spindle to oval cells of the epithelial nests, in our opinion, are another evidence for osteofibroblast origin. Based on our immunohistochemical demonstration of basal lamina materials together with the previous report on ultrastructural description of OFD-like adamantinoma,[2] we expected that the presence of basal lamina in the ultrastructural study. Unexpectedly, the basal lamina components were detected at the extracellular matrix, which may be due to the processing artifacts such as the long decalcification. The fibrous cells of adamantinoma is believed to be of benign, while the epithelial component is malignant.[14] In addition, not only is the epithelial- mesenchymal transformation is needed but also the second genetic events, such as p53 protein alteration may also be needed for adamantinoma to acquire malignant potential. Attempts have been made to diagnose and predict the biologic behavior of this rare OFD-like adamantinoma by molecular analysis such as detection of chromosomal aberration, DNA ploidy, etc.[15] Besides the rarity of the incidence, the long decalcification and fixation process further interfere with the anal-

ysis of molecular cytogenetics.

In examining OFD-like lesion in the biopsy materials, the representative biopsy samples should be taken from the central portion as well as the wide excision is mandatory because such fibrous lesion can occasionally be seen in the periphery even in classic adamantinoma and OFD.

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