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Adamantinoma of the Tibia.

— Report of a Case with Findings of Ultrastructural and Immunohistochemical Studies. —

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ABSTRACT : A case of tibial adamantinoma in a one-year and ten-month old girl is reported. She had gait disturbance and her roentogenogram showed a well circumscribed radiolucent area in the tibia. Light microscopic examination showed the epithelial component of nests and pseudoglandular arrays in the loose fibrous connective tissue. We finally diagnsed the tumor as adamantinoma of the tibia based on roentogenographical and histological findings. Moreover, the nature of the neoplastic cells was studied by the immunohistochemical and electron microscopic methods. The adamantinoma is regarded as a unique neoplasm capable of differentiating into epithelial elements as well as mesenchymal ones. Detection of epithelial component is important to differentiate from similar disorders. The fibrous dysplasia-like lesion in stroma is interpreted as a part of the spectrum of mesenchymal differentiation.

INTRODUCTION

Adamantinoma of the long bones is an uncommon neoplasm. The histogenesis of the tumor has been debated since Maier first described it in 1900. The two major proposals, endothelial or epithelial origin have been advocated. More recently, however, ultrastructural and immunohistochemical studies have demonstrated convincingly the epithelial nature of nests and islands seen in the tumor. On the other hand, attention has been drawn to the peculiar features of stroma in the tumor such as a fibrous dysplasia-like lesion. We report a case of tibial adamantinoma in a one-year and ten-month-old girl, and review its histogenesis.

CASE REPORT

A one-year and ten-month-old female infant was admitted to The Mitsugi Gereral Hospital on May 27, 1986, because of gait disturbance. About twenty days prior to her admission, she had fallen down stairs. Roentogenogram showed a large radiolucent lesion in the diaphysis of the tibia (Fig. 1). The lesion was well circumscribed, osteolytic or cystic, and located predominantly in the cortex (Fig. 2). There were no particular findings on other laboratory examinations. A local excision was performed. The excised tumor was elastic hard, measuring 3.0×1.5×1.5cm. The cut surface was yellowish white and solid (Fig. 3). An auto-

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genous bone graft was bridged in the osseous defect. The postoperative course was uneventful and the patient remained well after 10 months.

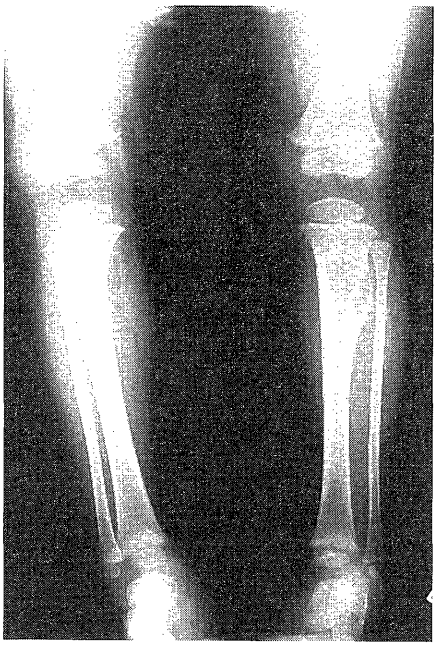

Fig. 1. This roentogenogram shows a well circumscribed radiolucent area in the proximal portion of the left tibia.

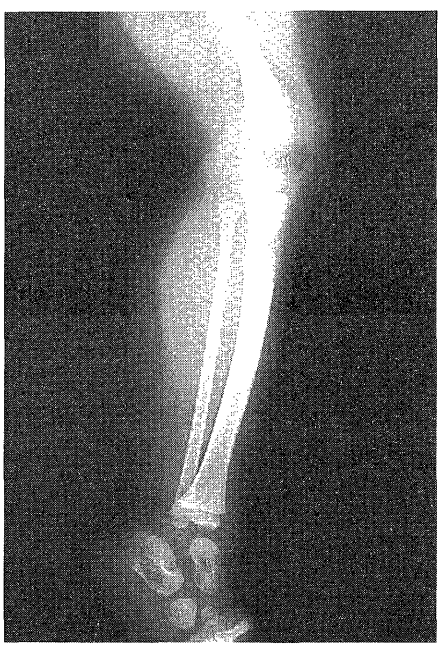

Fig. 2. This is a lateral view of the same lesion. Periosteal reaction is not evident.

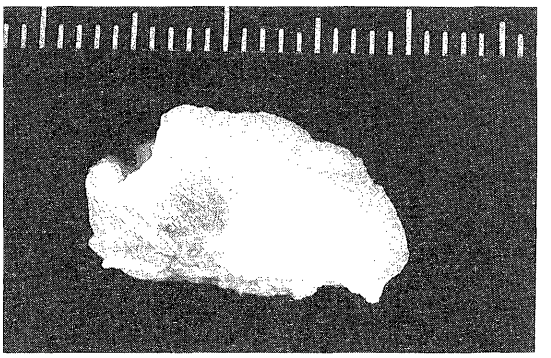

Fig. 3. Cut surface of this tumor is firm or rubbery in consistency, and pale gray to yellowish-white in colour.

MATERIALS AND METHODS

Light microscopic study

The specimen from the lesion was fixed in a solution of 10% formalin and embedded in paraffin without prior decalcification according to standard procedures. Sections were stained with hematoxylin-eosin, PAS, Hale colloidal Iron, Masson-trichrome, and silver stains.

Immunohistochemistry

Details of the antibodies and sera employed were shown in Table 1. Paraffin sections were stained by the peroxidase-anti peroxidase (PAP) method with some modifications. Briefly, sections dewaxed were treated with 0.3% solution of hydrogen peroxide in absolute methyl alcohol at room temparature to block endogenous peroxidase activity. After exposure to normal swine or rabbit serum to reduce nonspecific staining, the sections were incubated with the first antibodies overnight at 4 ℃. Then the sections were washed three times with 0.01 M PBS for 5 minutes and incubated with second antibodies for three hours at room temperature. They were washed again and incubated with peroxidase-anti peroxidase complex for one hour at room temperature. Then the sections were washed and treated for five minutes with 0.05% 3－3' diaminobenzidine and 0.005% H_2O_2 in tris-saline buffer solution at pH 7.4.

Negative controls included replacement of the primary antibodies with PBS, normal rabbit and normal mouse serum.

Electronmicroscopic study

Formalin-fixed blocks were dissected into one millimeter cubes, fixed again in 2.5% phosphate buffered glutaraldehyde,then washed with phosphate buffer for 12 hours, postfixed in 1.0% osmium tetroxide for one hour, dehydrated in a graded ethanol series, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEOL 100-B electron microscope.

Table 1. List of Antibodies and Sera used in This Study and Their Optimal Dilution.

1) Primary antibodies
- # Rabbit antiserum to human factor VIII related antigen, 1:100, DAKO.
- # Rabbit antiserum to human keratin, KIT, Kyowa.
- # Mouse antiserum to human vimentin, 1:100, DAKO.

2) Secondary antibodies
- # Swine anti-rabbit, 1:50, DAKO, : for the PAP methods.
- # Rabbit anti-mouse, 1:50, DAKO, : for the PAP methods.

3) PAI
- # PAP, rabbit, 1:100, DAKO.
- # PAP, mouse, 1:100, DAKO.

4) Others
- # Normal swine serum, 1:20*, DAKO.
- # Normal rabbit serum, 1:20*, 1:100**, DAKO
- # Normal mouse serum, 1:100**, CL.

* : for reduction of non-specific background staining.
** : for negative control staining.
DAKO : DAKOPATTS, Copenhagen, Denmark
Kyowa : Kyowa Medix Inc. Tokyo
CL : Cedarlane Laboratories, Ltd., Ontario, Canada.

RESULT

Light microscopic features

The tumor was composed of looose fibrous connective tissues with nests or sheets of epitheloid cells (Fig. 4). And a part of the epithelial component represented pseudoglandular arrays. A few large nests had irregular cleft-like lumina containing papillae of epithelial cells (Fig. 5). Also vessels were scattered in the fibrous matrix.

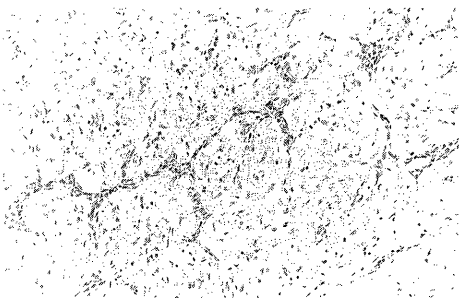

Fig. 4. This photomicrograph shows the nests or sheets of tumor cells in fibrous tissue. H.E. ×100.

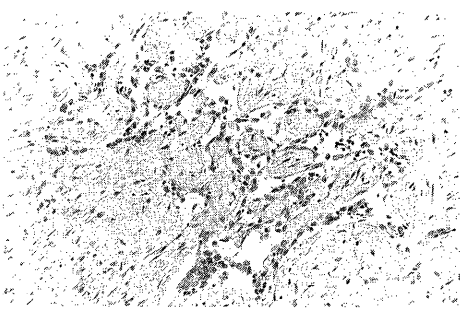

Fig. 5. Note a pseudoglandular pattern consisted of epithelial cells with an intervening fibrous stroma. H.E. ×100.

Cytologically the cells were oval or round in shape and were not markedly pleomorphic. The nuclei had a uniform vesicular chromatin pattern with single nucleolus. The cytoplasm was narrow and faintly eosinophilic. Hale colloidal iron and PAS stains were negative for intracytoplasm.

Some lymphocytes aggregation around perivascular lesion scattered in the abundant stroma. A fibrous dysplasia-like lesion was also present in the stroma, which was consisted of loose fascicles elongating in a vague striform pattern around blood vessels (Fig. 6). Trabeculae of woven bone were formed at the periphery of the tumor (Fig. 7). However. the lesion differed from the typical fibrous dysplasia in the presence of the epithelial islands. These epithelial islands gradually blended with fibrous background. In a part of the tumor, the stroma was abundant of collagen showing a sclerotic appearance.

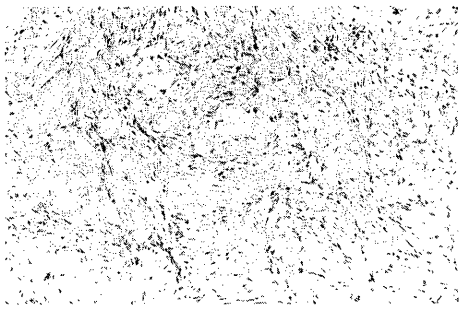

Fig. 6. This field shows part of a fibrous dysplasia-like lesion consisting of loose fascicles of fibroblasts. Tumor islands are not evident. H.E. ×40.

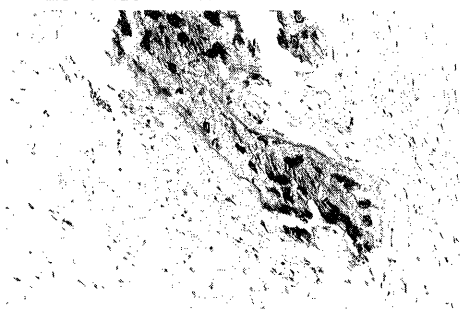

Fig. 7. Trabeculae of woven bone are formed directly from stroma at the periphery of the tumor. H.E. ×40.

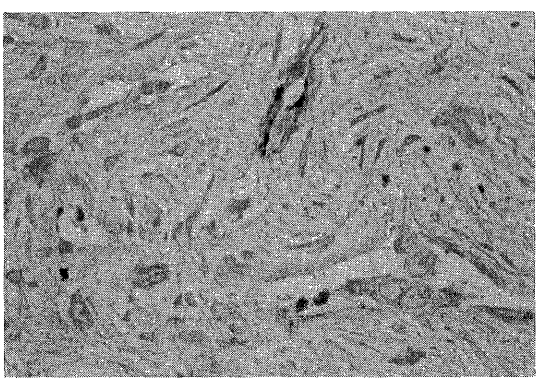

Fig. 8. Positive staining for factor VIII-related antigen in the endothelial cells of stromal vessels. The adjacent epithelial cells are negative. PAP stain. ×400.

Immunohistochemical features.

Stain for factor VIII-related antigen showed strong positivity in most of the endothelial cells lining the capillaries and venules in the stroma (Fig. 8), while all of the epithelial cells forming nests and channels were uniformly negative for this marker.

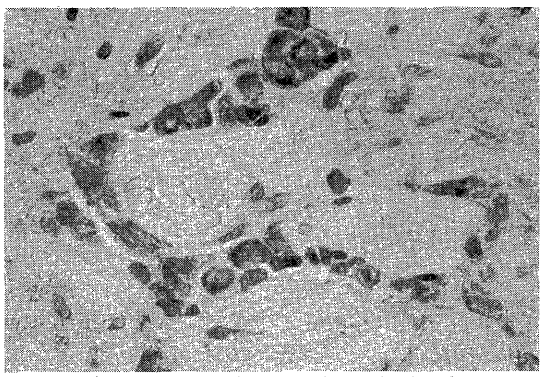

Fig. 9. Positive staining for keratin in the cytoplasm of the epithelial cells. PAP stain. ×400.

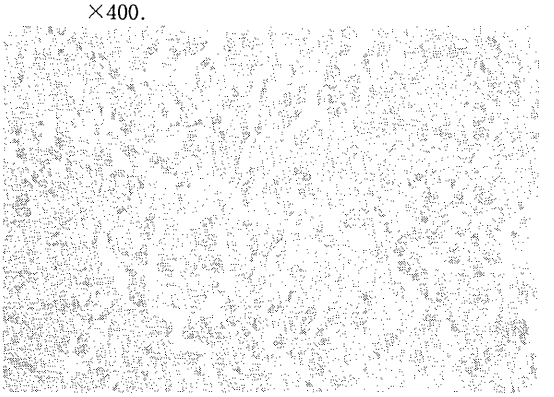

Fig. 10. Weakly positive staining for vimentin in the epithelial and stromal cells. PAP stain. ×200.

Keratin was stained intensely in the cytoplasm of all the cells presented in the nests and channels (Fig. 9), while the endothelial cells of blood vessels and other stromal elements remained negative. Furthermore, keratin was stained in the perinuclear narrow cytoplasmic rim. On the other hand, the stain for vimentin was weakly positive in the epithelial and stromal elements (Fig. 10).

Electronmicroscopic features

Some details were obtained by electronmicroscopic observation. The tumor cells had irregularly folded nuclear membranes with unevenly dispersed chromatin. The cytoplasm of these cells had numerous cytoplasmic projections and tightly interdigitated microvilli, and small intercellular lumina were formed by these cells (Fig.11). Desmosome-like structures were also seen (Fig. 12). The striking feature

Fig. 11 Microvillous projections are seen in the intercellular lumen(L) bounded by cells. Intercellular junction apparatuses are seen(arrow). Uranyl acetate and lead citrate. ×23,000.

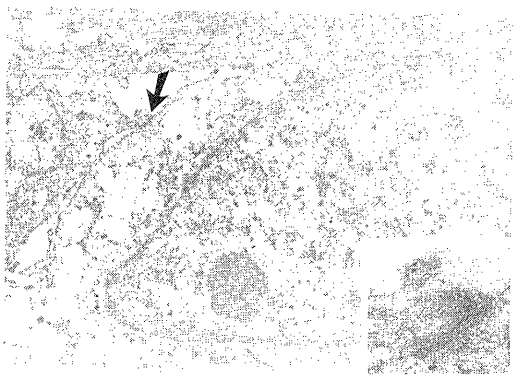

Fig. 12. Desmosome-like junction connecting two tumor cells is seen(arrow). Uranyl acetate and lead citrate. ×11,500. Higher magnification. ×62,000.

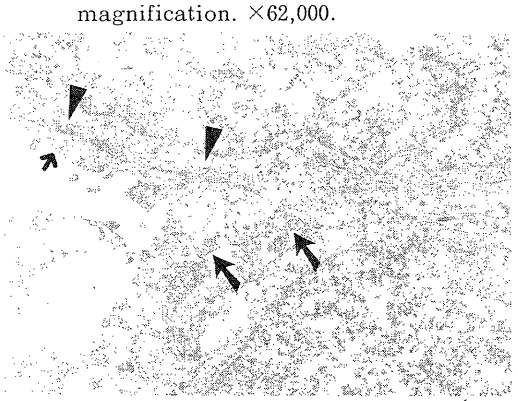

Fig. 13. Incomplete-formed basal lamina (short arrows), tonofilaments (arrowheads) and cell processes (long arrows) were indicated. Uranyl acetate and lead citrate. ×18,400.

was the presence of tonofilaments and tonofibrils, which were composed of microfilament bundles with frayed ends (Fig. 13). Cytoplasm contained a few of disturbed mitochondrias, rough endoplasmic reticulums and small vesicles. Basal lamina was found out. In the stroma, fibroblast-like cells which contained numerous filamentous materials were obserbed. Capillaries were seen as well there.

DISCUSSION

Adamantinoma of long bone is a rare distinctive tumor, which occurs principally in the tibia. Since this neoplasm was first described by Maier in 1900[12], approximately over 160 cases have been reported in the English and Japanese literatures. The detailed statistics and clinical events has been gradually elucidated[8)14)15)22)]. Nevertheless, the histogenesis of this neoplasm is still controversial. Vascular[4)6)8)11)], synovial[10)16)] and epithelial structures[2)9)17)18)19)20)24)] have been proposed as cellular origin.

Histologically, small epithelioid islands are found in fibrous stroma. Relative amount of two components vary considerably. The epithelioid islands vary in structure and shape case by case. Four different epithelioid patterns are present in adamantinoma[3)23)] : spindled, basaloid, squamoid, and tubular. These various histologic features have led to the continuing controversy over its histogenesis. Currently, it is believed that this tumor is an epithelial origin by ultrastructual findings or immunohistochemistry. Our immunohistochemical and ultrastructural studies suggested the differentiation into the epithelial element. Our findings confirmed those of ROSAI *et al.*[18] or KNAPP *et al.*[9], that is to say, keratin was stained strongly in the almost all of the epitheloid cells. Our ultrastructual features demonstrated cytoplasmic processes and projections of microvillous membrane. In addition, basal lamina, desmosome-like structures and tonofilaments with frayed ends could be seen. We are unable to support a vascular origin for adamantinoma, because we could not find out Weibel-Palade bodies and proliferation of tumoral vessels.

It is also well known that keratin proteins are more wildly distributed in biologic system than formerly thought[21], and it does not necessarily express squamous character only. Certainly the presence of highly differentiated keratinizing squamous epithelium as previously reported should not be used as irrefutable evidence of epithelial origin, since synovial sarcoma, a tumor generally accepted as mesenchymal, may also have areas of keratinizing squamous epithelium and mesothelium may undergo squamous metaplasia in respose to various stimuli[23].

The epithelial component is a clue to diagnosis of adamantinoma, but investigation of stromal component composed of spindle cells is also important in view of histogenesis of the tumor. The fibrous areas either may represent a part of the tumor or may be a reaction. Areas that suggest transition between the fibrous and epithelial areas are indicative of the former possibility. The stroma in our case was consisted of fibroblast-like cells and it resembled fibrous dysplasia. Therefore, the tumor appears to differentiate along both mesenchymal and epithelial lines. Our results were consistent with the features of synovium or mesothelium[7]. We believe that origin of adamantinoma would be the cell having the biphasic potentiality. Nevertheless, it is interesting that, recently, BAMBIRRA *et al.*[1] and MILLS *et al.*[13] described some tumors morphologically identical to tibial adamantinoma located not in the bone but in the skin and soft tissue over-lying the anterior surface of the tibia. Moreover, EINSTEIN *et al.*[5] suggested the idea that tibial adamantinoma was a form of eccrine carcinoma by using enzyme histochemistry. Their explanation was different from ours, because they doubted whether the stromal component would be neoplastic.

In any event, although several thories on histogenesis have been proposed, it would be premature to limit the origin to peculiar tissues such as epidermis or skin appendages. To determine the correct origin and the exact mechanism of growth on tibial adamantinoma, additional cases of this entity should be studied in detail.

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