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—Case Report—

ULTRASTRUCTURE OF BOTRYOID SARCOMA OF THE COMMON BILE DUCT

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Summary

A case of botryoid sarcoma of the common bile duct in a 4-year-old girl was reported. Electron microscopic examination disclosed that the neoplasm consisted of three types of cells: polygonal, elongated, and small cells. The former two contained moderate to large amounts of poorly developed myofibriles in the cytoplasm with occasional A, I and Z-bands. The small cells contained mitochondria and dilated rough endoplasmic reticulum but few myofilaments. Deposits of glycogen granules were constant components of the neoplastic cells. Mitosis was striking in the small cells. Abnormal multilaminar endoplasmic reticulum was observed in the small cells in the mitotic stage.

Key Words: *botryoid sarcoma, choledochus, myofibrile, myofilament.*

Botryoid sarcoma (rhabdomyosarcoma) of the common bile duct is a rare malignant neoplasm occurring only in infants and children[5,9,10,17]. The ultrastructure of rhabdomyosarcoma of the orbit[3,12,13], ear[8], prostate[16], diaphragm[6], and limb muscles[4,11] has already been reported, but that of the common bile duct has not yet been reported.

In the present study, the ultrastructure of cytotopical characteristics of botryoid sarcoma of the common bile duct in a 4-year old girl was examined and compared with the rhabdomyosarcoma of other organs in man and in myogenetic studies in experimental animals[1,7].

Case Report

A 4-year-old Japanese girl was admitted to the Nagasaki University Hospital, on July 15th 1975, with complaints of chills, fever, intermittent abdominal pain and jaundice. Physical examination revealed a well-nourished, normally developed but deeply jaundiced child, suffering from hepatomegaly. Laboratory examination revealed: serum bilirubin, 7.5 mg/dl (direct 6.2 mg/dl, indirect 1.3 mg/dl); alkaline phosphatase, 11.4 K.A. units; total protein, 8.1 g/dl; SGOT, 93 units; SGPT, 63 units; thymol turbidity, 3+; cephalin flocculation, negative. Stool was positive for occult blood. Urinalysis indicated a brown, cloudy urine with albumin which was strongly positive for bile.

Body temperature was elevated to 39.5°C and abdominal pain continued throughout the preoperative days, although the patient was treated with antibiotics. Other abnormalities included a persistent elevation of bilirubin, a rising alkaline phosphatase and absent urinary urobilinogen. A laparotomy was performed on October 22nd.

Surgical findings revealed an enlarged liver and distended gall bladder. There was a mass in the hepato-duodenal ligament which resembled a choledochal cyst. An incision was

made into the common bile duct, where a large volume of soft, friable tissue was discovered. A cholecystectomy with resectioning of both the common hepatic and bile ducts and anastomosis of the hepatic duct confluent to the defunctionalized jejunal loop were performed.

Materials and Methods

Tissues for light microscopy were fixed in 10% formalin and embedded in paraffin. Sections of paraffin embedded tissue were stained with H & E, phosphotungtic acid and hematoxylin stains. Tissues for electron microscopy were minced into 1 mm cubes, fixed in refrigerated, buffered glutaraldehyde for one day, washed in 0.1 molar phosphate buffer, postfixed in osmium tetroxide and embedded in epoxy resin. Ultrathin sections mounted on copper grids were examined and photographed with Hitachi-12, JEM-100B and 7A electron microscopes.

Results

Light Microscopic Findings:

Surgically removed tissue contained multiple polypoid lesions. The surface was lined with an intact single layer of bile duct epithelium. The underlying tissue varied in appearance; some areas were extremely edematous; others were very dense with cellular components. These dense areas were occasionally composed of randomly oriented spindle cells. The cytoplasm was generally scanty and nuclei were hyperchromatic. Cross striations in the cytoplasm were rarely visible. Numerous mitotic cells were observed (**Fig. 1**).

Electron Microscopic Findings:

The neoplasm consisted of three types of cells: polygonal, elongated, and small cells.

The polygonal cells had large nuclei with relatively irregular margins. The nuclear chromosomes were dispersed. The broad cytoplasm contained moderate amounts of readily identifiable myofilaments. These myofilaments consisted of both thick (approximately 150 Å) and thin (50 Å) types. The myofibrils, which were composed of several myofilaments, were oriented in different planes as demonstrated by oblique, longitudinal, and cross-sectioning (**Fig. 3, 4**). Myofibrils, which were arranged in more organized regions, were aligned in arrays parallel to each other and displayed darkly and lightly stained areas in the longitudinal sections. They manifested poorly formed banding which included a dense 1.5 μ A-band and a dense 0.1 μ Z-band within a light 0.5 μ I-band. Neither H nor M-bands, however, were clearly observed in the A-band. These bands of myofibrils were attributed to the special arrangements of thick- and thin filaments. The I-bands were composed of thin myofilaments and the A-bands were composed of thick and thin myofilaments (**Fig. 3, 4**).

The arrangements of interdigitating thick and thin myofilaments became more obvious in cross sections. The thick myofilaments were arranged in a hexagonal pattern, 450 Å apart, with six thin myofilaments around each thick myofilament. This band was considered to be the A-band (**Fig. 4**). Mitochondria and large aggregates of glycogen granules were observed in the cytoplasm.

The elongated cells had extremely long cytoplasmic processes and appeared more dense due to a greater number of myofilaments. The myofilaments tended to form arrays along the long axis of cells; sarcomere-like arrangements were more conspicuous. However, the special bandings could not be demonstrated unequivocally in the myofilaments. Occasionally, myofilaments were found in small aggregates reminiscent of poorly formed Z-bands (**Fig. 5**).

The small cells were quite different from the

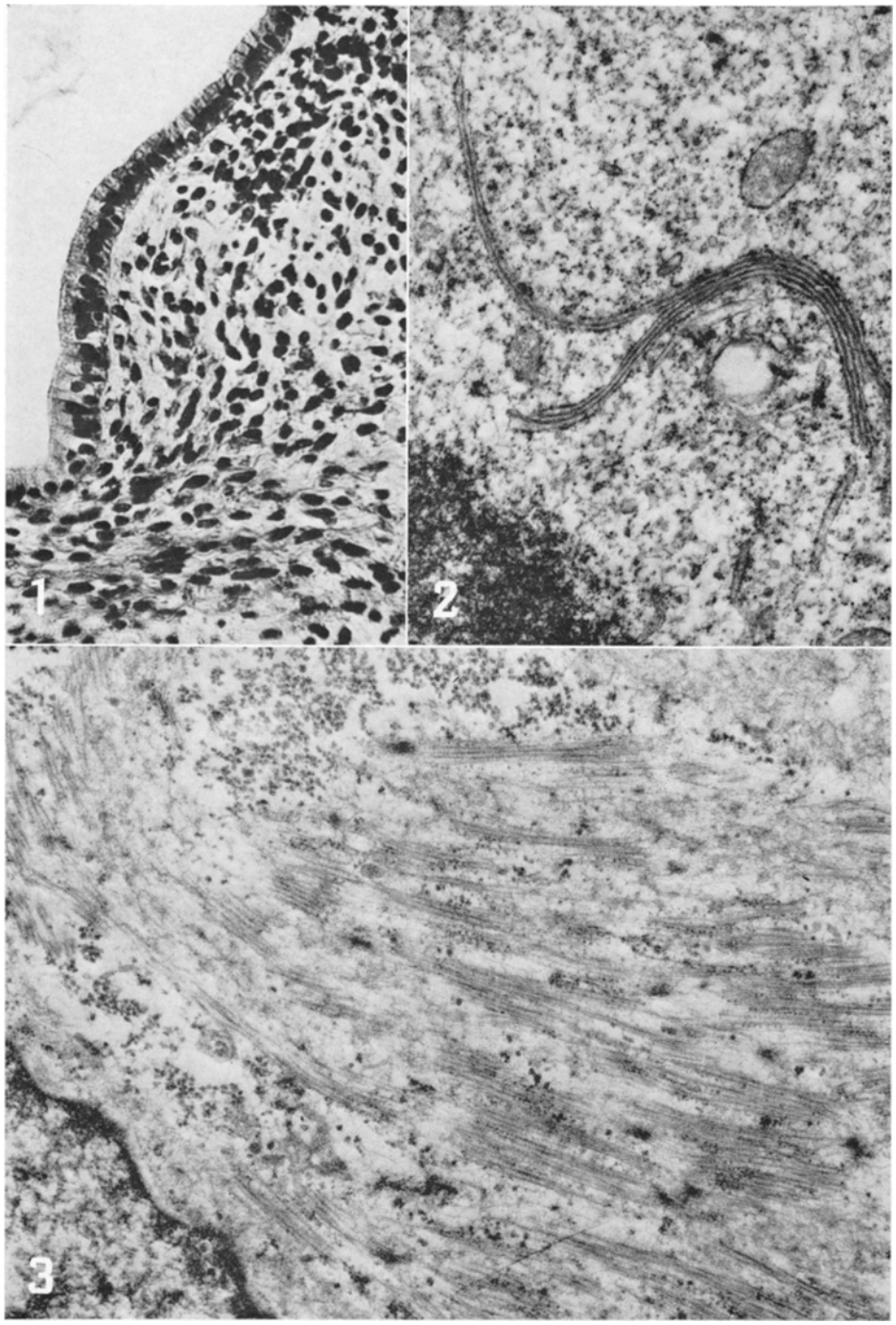

Fig. 1. Polypoid lesions (H & E stain). Neoplastic tissues are covered by a single layer of intact bile duct epithelium and are composed of spindle shaped cells which run in rudimentary directions. ×600

Fig. 2. Small cell in anaphase. Membranes stacked 10 layers are visible in the multilaminar endoplasmic reticulum (MER). One MER has separated ntotwo. The external membranes of MER are attached to ribosomes. The nner membranes are smooth. The MER is connected to the rough endoplasmic reticulum. ×18,000

Fig. 3. Polygonal cell. Many myofibriles display electron dense A-bands and light I-bands with dense Z-bands in the longitudinal sectioning. ×18,000

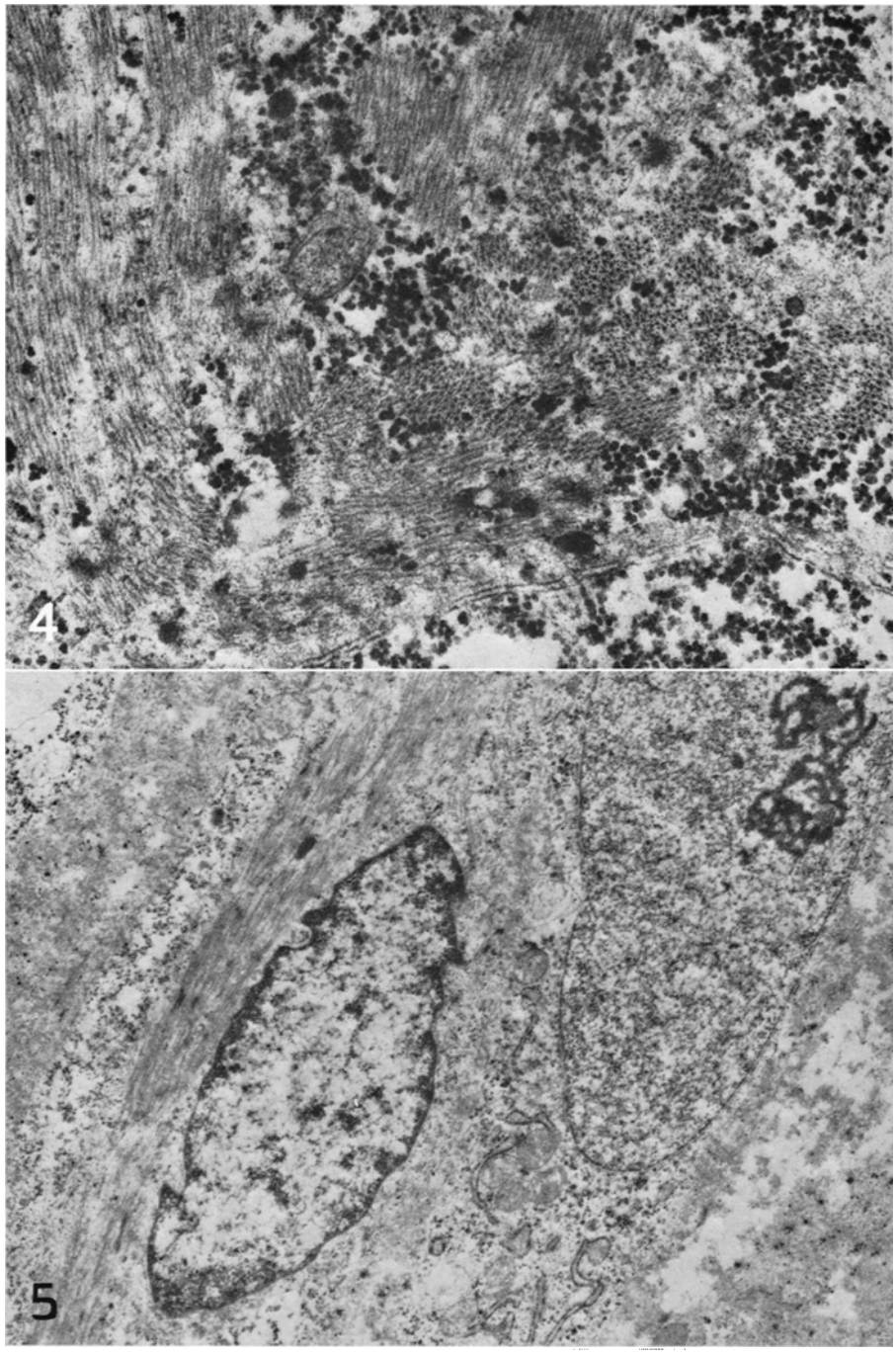

Fig. 4. Polygonal cell. The myofibriles are composed of thick and thin myofilaments apparent in longitudinal and cross sectioning. ×30,000

Fig. 5. Elongated and small cells. An elongated cell has many myofilaments which are arranged along the long axis of the cells. Occasional dense areas are Z-band suspects. A small cell has many mitochondria and well-developed rough endoplasmic reticulum. ×14,000

former two cell types. Their nuclei were relatively large and oval shaped, and the chromatin was dispersed. Nucleoli were prominent. Narrow cytoplasm contained moderated amounts of mitochondria, glycogen granules, well-developed endoplasmic reticulum and Golgi apparatus, but few myofilaments (**Fig. 5**). The small cells contained high levels of mitotic activity. Multilaminar endoplasmic reticulum (MER) composed of 4, 6 or 10 stacked membranes was noted in all mitotic cells (**Fig. 2**).

Discussion

Electron microscopic examination of botryoid sarcoma provides a much greater diagnostic capability since it reveals immature striations in neoplastic cells not capable of resolution by light microscopy.

In the present study, botryoid sarcoma consisted of three types of cells: polygonal, elongated and small cells. Polygonal cells contained poorly formed A, I and Z-bands in the myofibrils. The elongated cells also contained many myofilaments but banding was relatively obscure; These cells were easily identified as skeletal muscle cells[2,15]. The small cells contained few myofilaments.

Several ultrastructural studies of rhabdomyosarcoma in other organs have been reported. The ultrastructural characteristics of rhabdomyoma cells containing myofilaments were basically similar at all sites[3,6,8,11,12,13,16]. Kroll et al.[13] reported two cases of orbital rhabdomyosarcoma. They observed myofilaments with A, H, M, I and Z-bands in the sarcoma cells only in the well-differentiated type and not in the embryonal type. Sarber[16] reported sarcoma cells consisting of three types of cells: large polygonal, elongated fiber-like and small cells in rhabdomyosarcoma of the prostate. The former two contained myofilaments with Z-bands and few myofilaments in the small cells. Friedman[8] reported myofilaments consisting of A, I and Z-bands in a case of rhabdomyosarcoma of the ear, which were analagous to our case.

Interesting ultrastructural studies of myogenesis of embryonic rat[1] and chick[7] skeletal muscle cells have been reported. They found that developing myoblasts contained poorly formed Z-bands in the myofibriles. These electron micrographs revealed a striking resemblance between embryonic skeletal muscle cells and polygonal and elongated cells in the botyoid sarcoma reported above. The very youngest myoblasts of embryonic skeletal muscle cells resemble the small cells described in our report. A possible explanation for this resemblance might be that the more random the alignment of myofilaments, the more poorly differentiated the cell.

Mitotic activity was striking in the small cells. These observations indicated that the small cells were the most malignantly active. In the mitotic stage, various abnormal endoplasmic reticulum was observed. The multilam nar endoplasmic reticulum consisted of 4 stacked membranes and was reported in Hodgkin's cells[14], but there have been no reports of membranes stacked n 6 or 10 layers as observed in our study during anaphase and beginning telophase as shown in **Fig. 5**.

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References

1) Bergman RA: Observation on the morphologenesis of rat skeletal muscle. Bull Johns Hopkins Hosp 110: 187, 1962
2) Bloom W, Fawcett DW: A textbook of histology, Tenth edition, p. 295. W.B. Saunders Company, Philadelphia, London, Toronto, 1975
3) Chapman GB, Jones IS, Spelberg WW: An electron microscope study of rhabdomyosarcoma. Invest

Ophthal 2: 538, 1967
4) Corbeil LB: Differentiations of rhabdomyosarcoma and neonatal muscle cells in vitro. Cancer 20: 572, 1966
5) Delany MH, Driscoll PJ, Ainsworth H: Sarcoma botryoides of the common bile duct. J Pediat Surg 1: 571, 1966
6) Ebihara Y, Kitazawa Y, Shoda H, Koya N, Shike S: An autopsy case of embryonal rhabdomyosarcoma, light and electron microscopic observations. J Clin Electron Microscopy 7: 177, 1974
7) Fishman DA: An electron microscope study of myofibril formation in embryonal chick skeletal muscle. J Cell Biol 32: 557, 1967
8) Friedman E, Harrison DFN, Tucker WN, Bird ES: Electron microscopy of rhabdomyosarcoma of the ear. J Clin Path 18: 63, 1965
9) Hays DM, Snyder WH Jr: Botryoid sarcoma of the bile ducts. Amer J Dis Child 110: 595, 1965
10) Horn RC Jr, Yokovac WC, Kaye R, Koop CE: Rhabdomyosarcoma of the common bile duct. Cancer 8: 468, 1955
11) Horvat BL, Caines M, Fisher ER: The ultrastructure of rhabdomyosarcoma. Am J Clin Path 53: 55, 1970
12) Imaizumi M, Nukada T, Okada F, Abe H: Fine structure of rhabdomyosarcoma. J Electron Micro 23: 124, 1974
13) Kroll AJ, Kuwabara T, Howard GM: Electron microscopy of rhabdomyosarcoma of the orbit. Invest Ophthal 2: 523, 1963
14) Parmley RT, Spicer SS, Garvin AJ: Multilaminar endoplasmic reticulum and abnormal mitosis in Hodgkin tumor cells. Cancer Res 36: 1717, 1968
15) Rhodin JAG: Histology, a text and atlas, p 221. New York, Oxford University Press, London, Toronto, 1974
16) Sarber K, Tomnai G, McKay DE: Embryonal rhabdomyosarcoma of the prostate. Cancer 31: 442, 1972
17) Soper RT, Dunphy DL: Sarcoma botryoides of the biliary tree. Surgery 63: 1005, 1968

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