Rhabdomyoma

Report of Case with Ultrastructural and Histochemical Studies

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The benign tumor of striated muscle, or so-called rhabdomyoma, is a rare entity. The literature yields only 13 acceptable cases, if one excludes the cardiac lesion bearing the same name, and excludes as well a variety of other tumors which have occasionally been reported as rhabdomyomas. The present report deals with an additional case of a rhabdomyoma upon which, for the first time, histochemical enzyme reactions and ultrastructural studies were performed. These studies have helped to clarify the relationship of rhabdomyoma to normal skeletal muscle and to granular cell myoblastoma, as well as to the cardiac rhabdomyoma.

Report of Case

R. S., a 39-year-old white man, noticed a lump on the left side of the soft palate 6 years prior to admission to the Hospital of the University of Pennsylvania. At that time the mass was excised (presumably not completely) at another hospital. Five years later the mass recurred and steadily increased in size.

On his admission to the University hospital, significant physical findings were limited to the area of the tumor which involved the soft palate and pharyngeal wall on the left. The mucosa of this region was raised but not ulcerated. Externally a 5- by 6-cm. mass was palpable at the angle of the left mandible and submandibular space. There was no palpable cervical lymphadenopathy and the trachea was in the midline. Following a biopsy, resection of this mass was performed.

At operation it was noticed that most of the tumor was retropharyngeal and multilobular. It extended as far as the jugular foramen. The tumor was excised and the patient was discharged in good condition. The patient is living without recurrence 12 months after the operation.

Pathologic Features

Gross findings. The specimen consisted of multiple brown lobulated pieces of tissue weighing 95 Gm. in toto. Cross-sections showed uniform yellow-brown fleshy tissue with a few foci of necrosis (Fig. 1).

Microscopic findings. Sections (Figs. 2 to 4) revealed the tumor to be composed of large round to oval cells with pale, pink-staining, faintly granular cytoplasm. Many cells contained large vacuoles. In a few cells definite cross-striations were identified. Isolated cells contained collections of crystallike particles, haphazardly arranged. The nuclei were uniform, round or oval, and vesicular, with fairly prominent nucleoli, and were usually centrally located. In a portion of the tumor close to an area of necrosis the cells appeared smaller, with somewhat more hyperchromatic nuclei. A few connective tissue septae with blood vessels traversed the tumor.

The slides from the first resection of the lesion which was performed at another hospital were reviewed and showed similar histologic features.

Materials and Methods

Tissue from the rhabdomyoma selected for hematoxylin and eosin stain, McManus-periodic acid-Schiff (PAS) reaction, Gomori’s iron stain, oil red O, Mayer’s mucicarmine, crystal violet, a modified Snook’s reticulum, and Masson’s trichrome stains was fixed in 10% neutral buffered formalin. The tissue

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June 1968  RHABDOMYOMA  783

Fig. 1. Gross appearance of cut surface of rhabdomyoma

selected for Bauer-Feulgen glycogen stain was fixed in Carnoy’s fluid. Zenker’s solution was used as a fixative for Mallory’s phosphotungstic acid hematoxylin (PTAH). The sections stained with PAS and Bauer-Feulgen glycogen stain were examined with and without antecedent diastase digestion. The oil red O stain was done on frozen section material; tissues prepared for the other stains mentioned above were embedded in paraffin.

Histochemical studies were performed on tissues obtained within 10 min. after excision. Those selected for acid phosphatase, alkaline phosphatase, and esterase reactions were immediately placed at 4 C. in a solution of calcium-formol. Adenosine triphosphatase (ATPase) and succinic dehydrogenase reactions were performed on material freshly frozen in liquid nitrogen. All the tissues prepared for histochemical reactions were cut in a cryostat at 6 μ.

The acid phosphatase method used has been reported by Evans and associates. The alkaline phosphatase determination was by Tsou’s modification of the acid phosphatase technic. Tsou’s modification of Barnett and Seligman’s indigogenic method for esterase was used. ATPase determination was by Padykula and Herman’s modification of Comori’s method, and succinic dehydrogenase determination was performed as described by Nachlas and colleagues.

In addition to tissue from the rhabdomyoma upon which all of the above named histochemical studies were performed, acid and alkaline phosphatases, esterase, ATPase, and succinic dehydrogenase were also studied in normal skeletal muscle. Tissue from a granular cell myoblastoma was studied for acid phosphatase and esterase activity.

Tissue from the rhabdomyoma for electron microscopy was prepared as follows. Small blocks of tissue away from areas of obvious necrosis or hemorrhage were fixed for 2 to 2 1/2 hr. at 4 C. in 5 % glutaraldehyde in 0.2 M cacodylate buffer and then washed during the next hour in three changes of 0.1 M cacodylate buffer with 10 % sucrose, also at 4 C. Tissue was then postfixed in Millonig’s fixative containing 1 % OsO₄ for 1 hr., dehydrated in graded alcohols and propylene oxide, and embedded in English Araldite. Sections stained with uranyl acetate and lead citrate were examined with a Siemens’ Elmiskop I. Routine histologic examination was made of sections of the tumor cut from paraffin-embedded tissue and stained with hematoxylin and eosin. The dimensions of ultrastructural features here
Fig. 2. Photomicrograph of rhabdomyoma showing large round to oval cells with scattered vacuoles. Hematoxylin and eosin. X 125.

recorded represent the average of at least 10 measurements made on prints enlarged 3 to 10× from original negative plates.

Results

The results of the special stains and histochemical methods are tabulated in Tables 1 and 2 and can be summarized as follows. The rhabdomyoma cells contained much glycogen and a very small amount of lipid. Iron and mucin were absent. Stains for amyloid were negative. Reticulum fibers encircled individual cells, and connective tissue was scant. Cross-striations were well demonstrated with the PTAH stain. Acid phosphatase, alkaline phosphatase, and esterase showed weak activity. ATPase activity was marked in most cells. Succinic dehydrogenase activity was intense, especially at the periphery of the rhabdomyoma cells. Within the cytoplasm, succinic dehydrogenase-positive material occasionally exhibited a linear distribution (Figs. 5 and 6).

In normal skeletal muscle there was no acid or alkaline phosphatase activity. Esterase activity was weak in normal muscle and in the rhabdomyoma. ATPase showed marked activity in some fibers (Type II) and none or weak reaction in others (Type I). Sections stained for succinic dehydrogenase activity showed a well defined striated pattern with areas of strong (Type I) and weak (Type II) activity. Granular cell myoblastoma showed extremely intense acid phosphatase and somewhat less intense esterase activity in the granular cells.

It is of interest that, despite the difficulty of finding convincing cross-striations with

<table>
<thead>
<tr>
<th>Special Stains</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Glycogen (PAS and Bauer-Feulgen)</td>
<td>Positive</td>
</tr>
<tr>
<td>Without antecedent diastase digestion</td>
<td>Negative</td>
</tr>
<tr>
<td>With diastase digestion</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>Negative</td>
</tr>
<tr>
<td>Lipid</td>
<td>Small amount present</td>
</tr>
<tr>
<td>Mucin</td>
<td>Negative</td>
</tr>
<tr>
<td>Amyloid</td>
<td>Negative</td>
</tr>
<tr>
<td>Reticulum</td>
<td>Encircling cells</td>
</tr>
<tr>
<td>Trichrome</td>
<td>Scant connective tissue</td>
</tr>
<tr>
<td>PTAH</td>
<td>Occasional cross-striations</td>
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</table>

<table>
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<tr>
<th>Enzymes</th>
<th>Rhabdomyoma</th>
<th>Granular Cell Myoblastoma</th>
<th>Normal Skeletal Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>1+</td>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1+</td>
<td>Not done</td>
<td>0</td>
</tr>
<tr>
<td>Esterase</td>
<td>1+</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>ATPase</td>
<td>3+</td>
<td>Not done</td>
<td>1+ and 3+*</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>3+</td>
<td>Not done</td>
<td>1+ and 3+†</td>
</tr>
</tbody>
</table>

* Type I and Type II fibers.
† Type II and Type I fibers.

TABLE 1
SPECIAL STAINS IN RHABDOMYOMA

TABLE 2
ENZYME ACTIVITY (GRADED 0 TO 4+) IN RHABDOMYOMA, GRANULAR CELL MYOBLASTOMA, AND NORMAL SKELETAL MUSCLE
FIG. 3 (upper left). Appearance of irregularly distributed crystal-like particles in rhabdomyoma cell. Phosphotungstic acid hematoxylin. X 800.

FIG. 4 (upper right). Well defined cross-striations in rhabdomyoma cell. Phosphotungstic acid hematoxylin. X 800.

FIG. 5 (lower left). Marked ATPase activity in rhabdomyoma. X 190.

FIG 6 (lower right). Succinic dehydrogenase-positive material in rhabdomyoma concentrated at periphery of cells and demonstrating areas suggestive of linear arrangement. X 190.

785
the light microscope, almost all of the tumor cells observed with the electron microscope could be definitely identified as striated muscle. In most there were large numbers of myofibrils and myofibrillar fragments. Often these consisted of tufts of myofilaments attached to both sides of a single Z band or joining together two or three Z bands in units of irregular and varying size. Usually these fragments were oriented at random in the cytoplasm, and only occasionally were they well organized with a series of Z bands in more or less complete sarcomeres running in parallel. Such well organized areas would give the appearance of cross-striations by light microscopy, whereas in areas of random orientation, most of the isolated myofibrils were below the limits of resolution of the light microscope. A well organized area in which the Z bands exhibited good alignment is illustrated in Figure 7. The vacuoles seen by light microscopy contained abundant glycogen and occasional lipid droplets.

Occasional cells contained circumscribed, roughly circular foci in which structures interpreted as enlarged, irregular, and randomly oriented Z bands were present; these often reached dimensions as large as 1 by 5 μ (Fig. 8). Numerous thin myofilaments were inserted into these, although there was no evidence of organization into sarcomeres. Further details of the ultrastructure of this tumor are reported elsewhere.4

Discussion

The literature on rhabdomyoma was recently reviewed by Moran and Enterline,16 who found a total of 11 cases and reported one of their own. Since their report, an additional case of rhabdomyoma was published by Tsukada and Pickren.27 The present report brings the number of cases in the literature to 14.

Nine of the tumors were in men and five were in women. Age distribution ranged from 8 weeks to 81 years with no predilection for any given age group. Eleven of the 14 tumors were located in the head and neck region. The others were found in the axilla, perineal region, and chest wall. Duration of symptoms prior to removal of the lesion varied considerably from 11 months to as long as 30 or 55 years.

Ten of the patients were followed for various lengths of time, up to 6 years postoperatively, without recurrence or metastasis. In the patient reported here the lesion recurred 5 years after the initial operation, which most likely was not a total resection. He has been followed now for 12 months after the second, more extensive resection without evidence of recurrence.

The “crystals” within the rhabdomyoma cells noted on light microscopy in both this case and the case reported by Moran and Enterline16 were shown to correspond to the highly hypertrophied Z band areas observed in the electron microscope. The chemical nature of this Z band material is still unknown, but it may be tropomyosin or a closely related protein. These abnormal structures appear similar to the hypertrophied Z bands seen in nemaline myopathy10 and to a lesser degree in denervation atrophy of muscle.11 Mitochondria are seen at the cell periphery and in linear rows between myofibrils in the portions of the cell which show the highest degrees of organization. This arrangement correlates well with the distribution of succinic dehydrogenase seen in the histochemical portion of the study.

The foci of hypertrophied Z bands in rhabdomyoma have not been described in the few cases of rhabdomyosarcoma subjected so far to ultrastructural studies. Although in some areas the organization of the myofibrils approaches that of normal muscle, in other areas the degree of fragmentation and disorientation resembles more closely that described in well differentiated rhabdomyosarcoma by Kroll and associates14 and by Friedman and co-workers.8

As has been noted previously, the superficial resemblance of rhabdomyoma to granular cell myoblastoma is not indicative of any relationship between these tumors. No cross-striations have ever been found in a granular cell myoblastoma, and histochemical evidence also points to marked differences between these two lesions. Fisher and Wechsler7 have emphasized the striking differences between granular cell myoblastoma and normal striated muscle and have presented evidence for the neurogenic nature of the granular cell myoblastoma.
FIG. 7 (upper). Well organized area of the cytoplasm of a rhabdomyoma cell in which bundles of myofilaments (arrowheads) can be seen to join several Z disks (Z) in a succession of sarcomeric units which simulate normal muscle but display abnormal branching and variation in size and arrangement. Arrows, transverse tubules; M, mitochondria; G, glycogen. × 32,000.

FIG. 8 (lower). Cytoplasmic area corresponding to focus of "crystals" by light microscopy. These are seen to consist of structures (Z) interpreted as enlarged and modified Z bands. They are often attached to bundles of thin myofilaments (arrowheads). G, glycogen. × 11,000.
The rhabdomyoma of striated muscle and that of the heart bear more than a superficial resemblance. In both lesions cross-striations are present; both contain glycogen and both demonstrate vacuolization of cells. However, as has been pointed out by Moran and Enterline, the cardiac rhabdomyoma cells are larger, with a more uniform vacuolization than those of the rhabdomyoma of striated muscle. Cross-striations of the cardiac rhabdomyoma are numerous and appear compressed by the vacuoles, whereas in the extracardiac lesion the cross-striations are rare and difficult to find. The haphazardly arranged crystals constituting hypertrophied Z bands which can be observed in the extracardiac rhabdomyoma have not been described in the cardiac lesion. Although more than half of the cardiac rhabdomyomas reported have been associated with tuberous sclerosis, such an association has not been reported to date in any of the extracardiac rhabdomyomas.

There also may be enzymatic differences between the cardiac and extracardiac rhabdomyoma, as Golding and Reed reported that a cardiac rhabdomyoma demonstrated neither succinic dehydrogenase nor ATPase activity, although their failure to demonstrate ATPase may be due to the method used. Succinic dehydrogenase and ATPase activity were marked in the extracardiac lesion. Unfortunately, no ultrastructural study of cardiac rhabdomyoma has been published for comparison.

**SUMMARY**

This is a report of a rhabdomyoma of the retropharynx in a 39-year-old man. For the first time histochemical enzyme reactions and ultrastructural studies have been performed on this lesion. These studies served to confirm its origin from striated muscle. Ultrastructural studies have established that the "crystals" seen in extracardiac rhabdomyoma by light microscopy represent abnormal hypertrophied Z bands similar in size and type to those seen in nemaline myopathy. The tumor differs sharply in enzyme pattern and structure from both granular cell myoblastoma and cardiac rhabdomyoma.

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**REFERENCES**