Fine-structural classification of orbital rhabdomyosarcoma

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Six cases of orbital rhabdomyosarcoma were studied with the electron microscope. Tumor cells (rhabdomyoblasts) could be classified into three fine-structural types: (1) Myofibrillar cells had large amounts of cytoplasm containing characteristic well-differentiated banded myofibrils. Each myofibril consisted of thick and thin myofilaments grouped in parallel array. (2) Myofilamentous cells had moderate amounts of cytoplasm containing thick and thin myofilaments in poorly ordered array with indistinct banding and little tendency toward myofibril formation. (3) Nonspecific cells usually had small amounts of cytoplasm with a few widely scattered, randomly oriented thin filaments only. There was no tendency in this cell type to form filament aggregates. It is suggested that electron microscopy may contribute to the specific diagnosis of rhabdomyosarcoma by detecting actomyosin filaments which could not be detected with light microscopy. If possible, the histology of an orbital biopsy should be supplemented by a fine-structure study when rhabdomyosarcoma is being considered in the differential diagnosis.

Rhabdomyosarcoma, the most common primary malignant orbital tumor of childhood, has been classified histologically as embryonal, differentiated, or alveolar. In a previous report, the fine structure of two tumors, one differentiated type and one embryonal type was described. A detailed study of four additional cases with phase-contrast and electron microscopy now permits a tentative classification of tumor cell (rhabdomyoblast) type based upon fine-structural characteristics.

Materials and methods

Tumor tissue was obtained at time of surgery from 6 children with orbital rhabdomyosarcoma (Table I). The ages ranged from 3 to 7 years with an average age of 5. There were 3 boys and 3 girls. One tumor was histologically classified as differentiated and the other five embryonal. No alveolar type tumors were found.

The tissue samples were cut in small pieces with a sharp razor blade and fixed in either 2 per cent buffered osmium tetroxide or 4 per cent buffered glutaraldehyde followed by osmium tetroxide. They were then dehydrated and embedded in Epon 812. Thick (2.5 μ) sections were cut and stained with paraphenylenediamine for phase-contrast microscopy. Serial-thin (0.05 μ) sections for electron microscopy were cut from the same block to correlate the phase microscopy and fine structure of a given cell. The thin sections were stained with uranyl acetate and lead citrate and examined in a JEM-7 electron microscope. At least two, and as many as six blocks from different areas of each tumor were sectioned and examined. Other portions of each tumor were fixed in formalin and processed for routine light microscopy.
Table I

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Orbit</th>
<th>Histology</th>
<th>Fine structure</th>
<th>Postoperative status</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. L.</td>
<td>7</td>
<td>M</td>
<td>R</td>
<td>Differentiated</td>
<td>Myofibrillar</td>
<td>Living and well, 4½ years</td>
</tr>
<tr>
<td>D. V.</td>
<td>5</td>
<td>F</td>
<td>R</td>
<td>Embryonal</td>
<td>Myofilamentous</td>
<td>Living and well, 1 year</td>
</tr>
<tr>
<td>M. D.</td>
<td>3</td>
<td>M</td>
<td>L</td>
<td>Embryonal</td>
<td>Myofilamentous</td>
<td>Living and well, 6 months</td>
</tr>
<tr>
<td>M. B.</td>
<td>5</td>
<td>F</td>
<td>R</td>
<td>Embryonal</td>
<td>Nonspecific</td>
<td>Living and well, 4 years</td>
</tr>
<tr>
<td>J. F.</td>
<td>5</td>
<td>M</td>
<td>L</td>
<td>Embryonal</td>
<td>Nonspecific</td>
<td>Deceased, 9 months</td>
</tr>
<tr>
<td>J. S.</td>
<td>4</td>
<td>F</td>
<td>L</td>
<td>Embryonal</td>
<td>Nonspecific</td>
<td>Deceased, 6 months</td>
</tr>
</tbody>
</table>

*Classification is based upon the most well-differentiated cell type found in the tumor with electron microscopy (see text).*

Results

**Myofibrillar cells.** In one case, classified histologically as differentiated and previously reported,² there were many tumor cells which had large amounts of cytoplasm (Fig. 1). Some of these cells had demonstrable cross-striations with Wilder's reticulum stain.²

With electron microscopy, the most differentiated cells in this tumor contained banded myofibrils (Figs. 2 and 3). Each myofibril contained both thick (approximately 150 Å wide) filaments and thin (approximately 60 Å wide) filaments. It is generally agreed that the thick filaments are myosin and the thin ones are actin.⁵ The filaments were grouped in parallel array and manifested the characteristic cross-banding of actomyosin. A dense 0.10 μ Z band was present within a lucent 0.50 μ I band, and a long 1.5 μ A band was bisected by a lighter 0.25 μ H band, which in turn contained a dense 0.05 μ M band. As in striated muscle in general, the I band contained only thin filaments; the A band both thick and thin filaments.⁶ Other cytoplasmic organelles present included mitochondria and ribosomes. Less well-differentiated cells, of the types described below, were also present in this tumor.

**Myofilamentous cells.** In the case described above and in 2 of the cases classified histologically as embryonal, there were...
Fig. 2. Myofibrillar cell cytoplasm, medium-power electron micrograph. The well-differentiated banded myofibrils consist of parallel aggregates of thick and thin myofilaments. Note that the myofilaments themselves are not in parallel array. Some are cut in longitudinal section, others in cross or oblique section. (Osmium tetroxide fixation. X26,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
Fig. 3. Myofibrillar cell cytoplasm, high-power electron micrograph. This is a portion of the field seen in Fig. 2. Note that the I band consists only of thin myofilaments, whereas the A band contains both thick and thin myofilaments. The dark Z band accounts for much of the banded appearance of actomyosin seen with light microscopy. Mitochondria and ribosomes are present. Ribosomes are the sites of cellular protein synthesis. (Osmium tetroxide fixation. x57,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
tumor cells with moderate amounts of cytoplasm which contained myofilaments in less well-ordered array (Figs. 4 to 8). In these cells, the myofilaments were aggregated into poorly defined groups which showed little tendency toward myofibril formation or banding. On occasion, an indistinct Z band could be recognized (Fig. 8). Mitochondria and ribosomes were also present.

**Nonspecific cells.** In all 6 cases there were tumor cells which were highly undifferentiated (Figs. 9 to 11). These cells had small amounts of cytoplasm which did not contain aggregates of specific myofilaments but merely a few widely scattered thin filaments which were randomly oriented (Figs. 10 and 11). There were also some mitochondria and ribosomes. The filaments may be actin filaments for they were occasionally found adjacent to better differentiated filament aggregates in some myofilamentous cells. However, at present, they must be regarded as nonspecific.

Based upon the most well-differentiated tumor cells found in each case with electron microscopy, one tumor could be classified myofibrillar, two myofilamentous, and three nonspecific (see Table I).

**Discussion**

From the data above, it is apparent that the degree of differentiation of a given rhabdomyoblast may be assessed by the fine structure of its cytoplasmic filaments. In the well-differentiated myofibrillar cells, the filaments form banded myofibrils. In the moderately differentiated myofilamentous cells, they form poorly organized filament aggregates. In poorly differentiated nonspecific cells, the filaments are not recognizable as actomyosin. It is interesting that the present fine-structural classification of this tumor is somewhat similar to the classification adopted by Ashton and Morgan\(^6\) based upon light microscopy: (a) embryonal sarcoma, completely undifferentiated, (b) nonstriated embryonal rhabdomyosarcoma where rhabdomyoblasts may be seen but no cross-striations found, and (c) striated embryonal rhabdomyosarcoma, where cross-striations can be demonstrated.

*Text continued on p. 540.*

**Fig. 4.** Myofilamentous cells, representative field, phase-contrast photomicrograph. These cells as a rule, contain less cytoplasm than myofibrillar cells. (Glutaraldehyde-osmium fixation. \(\times 400\).)
Fig. 5. Myofilamentous cell, low-power electron micrograph. A section of the entire cell is seen. In the cytoplasm are many myofilaments, ribosomes, and some mitochondria. (Glutaraldehyde-osmium fixation. ×8,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
Fig. 6. Same myofilamentous cell as in Fig. 5, high-power electron micrograph. There are thick and thin myofilaments in disordered array with no myofibril formation or banding. (Glutaraldehyde-osmium fixation. ×46,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
Fig. 7. Myofilamentous cell cytoplasm. medium-power electron micrograph. This cell, from the same tumor as Figs. 5 and 6, is somewhat better differentiated. The myofilaments tend to be parallel, have a slight tendency to form aggregates, and faint Z banding may be discerned on close inspection. However, true myofibrils are not present. Compare with Fig. 2. (Osmium tetroxide fixation. x30,000.) A, A band; f, thin myofilament (actin); F, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
Fig. 8. Same myofilamentous cell as in Fig. 7, high-power electron micrograph. Parallel thick and thin myofilaments are easily distinguished. Many ribosomes are present. In two areas faint Z banding in oblique section is seen, but no true myofibrils are present. Compare with Fig. 3. (Osmium tetroxide fixation. ×89,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
There is a possible objection to classifying a given tumor depending upon the most well-differentiated cell found with electron microscopy. The portions of tumor taken for fine-structural study are, of necessity, quite small, measuring only 1 or 2 mm square. An entire tumor, therefore can only be sampled rather than examined fully. It is perfectly possible that more extensive sampling and further study in a given tumor could have revealed better differentiated cells, and altered the ratio of tumor types in this series. In actual practice, however, this was not found to be true. Experience with six tumors has shown that the classification of a given tumor on initial examination was not altered by examination of as many as five additional blocks from different areas of the tumor.

Whether indeed the degree of differentiation of rhabdomyoblasts is of importance is also open to argument. Histologically, although cross-striations may be absent in the primary tumor, they may be present in recurrences or metastases, and vice versa. Finally, although some authors feel that histologic appearance may have prognostic significance, others disagree. In the present group of 6 patients, both of the 2 children who died from rhabdomyosarcoma had the least differentiated, nonspecific type tumor by electron microscopy. Of course, no prognostic conclusions should be drawn from such a small series.

Where then, might electron microscopy prove useful? The specific diagnosis of rhabdomyosarcoma with light microscopy is often difficult. The tumor may appear histologically as a highly undifferentiated malignancy of doubtful origin. A prolonged search is often required to demonstrate convincing cross-striations, and indeed a misdiagnosis of neurogenic sarcoma may be made. To characterize the cytoplasm of poorly differentiated cells, the use of Masson trichrome stain (which stains myoglobin brick red) or the use of PAS (to reveal diastase-sensitive PAS positive material) has proved helpful, but special stains are rarely of great help in demonstrating cross-striations.

From the fine-structural point of view, it is easily apparent why few tumor cells would be found with cross-striations. Not only must the cell be myofibrillar type with well-delineated Z bands, but the myofibrils within it must be lined up Z band to Z band across the cell to provide histolog-
Fig. 10. Nonspecific cells, low-power electron micrograph. The cytoplasm contains a few widely-scattered thin filaments which have no tendency to aggregate. (Osmission tetraoxide fixation. x16,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament myosin; H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
Fig. 11. Nonspecific cells, high-power electron micrograph. The filaments do not resemble the myofilaments of myofibrillar or myofilamentous cells. Compare with Figs. 3, 6, and 8. They must at present, be regarded as nonspecific. (Osminum tetroxide fixation. x33,000.) A, A band; f, thin myofilament (actin); ff, thick myofilament (myosin); H, H band; I, I band; L, lipid droplet; M, M band; m, mitochondrion; mf, myofibril; n, nucleus; r, ribosome; Z, Z band. Mark on the micrograph is equivalent to 1 μ.
ically visible cross-striations. Even in well-
differentiated myofibrillar cells (Fig. 2),
there is a limited degree of myofibrillar
alignment. Certainly, myofilamentous cells
whose Z bands are barely visible with
electron microscopy, and nonspecific cells
which contain no banded material, could
not have histologically visible cross-stria-
tions.

It would seem, then, that the electron
microscopist may have the potential for
demonstrating actomyosin filaments which
could not be detected with light micros-
copy, especially in myofilamentous type
cells. This would permit a definitive diag-
nosis of rhabdomyosarcoma. Because of the
small sample involved, however, a negative
statement would have much less signif-
icance.

In actual practice, electron microscopy
was found particularly useful in one of the
cases reported (M. D., Table I). In this
case the initial orbital biopsy was com-
patible with an undifferentiated malign-
nancy, but electron microscopy revealed
many myofilamentous rhabdomyoblasts,
making the specific diagnosis of rhabdo-
myosarcoma. Subsequent orbital exentera-
tion confirmed this diagnosis. Accordingly,
the author suggests that the histology of an
orbital biopsy be supplemented by a fine-
structure study when rhabdomyosarcoma
is being considered in the differential diag-
nosis.

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Boston, Massachusetts. The author acknowledges
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REFERENCES
3. Luft, J. H.: Improvements in epoxy resin embed-
5. Tice, L. W., and Barnett, R. J.: Fine-struct-
7. Hogan, M. J., and Zimmerman, L. E.: Ophthal-