Mesenchymal and Muscle-specific Intermediate Filaments (Vimentin and Desmin) in Relation to Differentiation in Childhood Rhabdomyosarcomas

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Twenty-one childhood rhabdomyosarcomas were divided into three groups on the basis of cyto logic composition. The tumors in group P consisted entirely of primitive mesenchymal cells, whereas those in groups M and W were characterized by the additional presence of numerous round rhabdomyoblasts and strap cells, respectively. The tumors were studied for the universal mesenchymal intermediate filament vimentin, and for the muscle-specific intermediate filament desmin. Vimentin positivity, which tended to be more prominent in primitive tumor cells, was found in all tumors, whereas desmin was found especially in round rhabdomyoblasts and strap cells. Desmin-positive primitive cells were found only in groups M and W, not in group P. It was concluded that the differentiation from primitive mesenchymal cells to morphologically recognizable myogenic tumor cells is accompanied by an increase in desmin positivity and, presumably, a decrease in vimentin positivity. Moreover, the observations suggest the existence of a group of "committed" cells that are morphologically primitive, but desmin-positive. These cells might play an important role in the observed further differentiation of rhabdomyosarcomas under chemotherapy (Hum Pathol 15:973, 1984). Hum Pathol 16:838–843, 1985.

Childhood rhabdomyosarcomas may feature a spectrum of different histologic presentations, as reflected by the designations alveolar, botryoid, embryonal, and pleomorphic rhabdomyosarcoma. They may also be subdivided, however, on the basis of cellular composition. In such a subdivision the tumors assumed to show the highest level of differentiation (group W) are characterized by the presence of numerous cells with long, extended eosinophilic cytoplasm (strap cells), with or without striations, that allow a definite light microscopic diagnosis of rhabdomyosarcoma. In the tumors assumed to be moderately differentiated (group M), a few strap cells may be found, but characteristically many large, round cells with strongly eosinophilic, fibrillary cytoplasm (round rhabdomyoblasts) are present. Although cross-striations are seen rarely in these cells at the light microscopic level, ultrastructural examination often reveals alternating thick and thin filaments and z-band material. In both groups primitive mesenchymal cells may be present as well. The tumors assumed to have the lowest level of differentiation (group P) are composed entirely of primitive mesenchymal cells, which lack myogenic characteristics at both the light and electron microscopic levels. A tentative diagnosis of rhabdomyosarcoma may be made on the basis of certain combinations of histologic and clinical presentations, e.g., alveolar sarcoma in the extremities of older children or young adults and botryoid sarcoma in mucosa in young children.

The development of antibodies directed against muscle proteins (e.g., myoglobin, myosin) and muscle-specific intermediate filaments (desmin) has added a useful tool for the diagnosis of rhabdomyosarcoma, especially poorly differentiated tumors. Several studies have addressed technical aspects and the use of different antibodies for diagnostic purposes. Little attention has been accorded, however, to the various cell types in rhabdomyosarcoma. Therefore, a group of childhood rhabdomyosarcomas was studied for the presence of the muscle-specific intermediate filament desmin and the universal mesenchymal intermediate filament vimentin. Differences in the staining characteristics of primitive mesenchymal cells, round rhabdomyoblasts, and strap cells were emphasized.

MATERIALS AND METHODS

Thirty specimens from primary or recurrent tumors in 23 patients were available for the study of intermediate filaments. One case was eliminated from the study because the available material was too scanty for reliable evaluation, and one additional case was eliminated because no staining was observed in tumor or surrounding tissue, presumably due to inadequate fixation. The remaining 21 cases were studied.

Formalin-fixed paraffin-embedded material was used for 25 specimens and material that had been snap-frozen in liquid nitrogen for two specimens (see table 2). One specimen was in part formalin-fixed and in part snap-frozen.

Antisera raised in rabbits against chicken gizzard muscle desmin and against vimentin isolated from calf lens were used. The specificity of desmin for muscle tissue had been evaluated previously in formalin-fixed paraffin-embedded normal and tumor tissues.

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For both vimentin and desmin labeling the peroxidase–antiperoxidase (PAP) technique was applied as described previously for paraffin-embedded and frozen tissue sections.20

RESULTS

Classification of Tumors

All cases were classified (table 1) according to cytologic composition4 (see introductory section). In group P, two tumors presented as classic alveolar sarcomas of the extremities in young adults and one as a botryoid sarcoma of the maxillary sinus in a child. The fourth tumor, an abdominal mass in an infant, appeared to be localized in the wall of the distal ileum. Microscopically, many "spider web cells"11 were found, leading to the diagnosis of rhabdomyosarcoma.

Intermediate Filament Studies

In all cases the numbers of cells that stained for desmin and for vimentin were estimated for each of the cell types present in the tumor (table 2).

Primitive cells. In all cases throughout the three groups, vimentin-positive primitive tumor cells were found. They varied in number from a few to virtually all of the cells present (fig. 1, top left). Desmin-positive primitive cells were found in all tumors in groups M and W (fig. 1, bottom left). The numbers of vimentin- and desmin-positive cells were not always easily compared, because many stromal cells, especially in botryoid sarcomas, also stained for vimentin. In the other histologic tumor types, however, the number of desmin-positive primitive cells was always less than that of vimentin-positive primitive cells, but the staining intensity of the former was usually stronger. In group P none of the formalin-fixed specimens showed any staining for desmin in tumor cells (fig. 1, top right), despite the staining for skeletal or smooth muscle observed in four of the six specimens. In case 4, however, staining was seen in many tumor cells in the frozen material.

Round rhabdomyoblasts. These cells, which were present in groups M and W, were generally positive for vimentin (fig. 1, bottom left). However, both the numbers of positive round rhabdomyoblasts and the intensity of their staining were often less than those of the primitive cells in the same sections. In most cases the majority of the round rhabdomyoblasts were strongly desmin-positive (fig. 1, bottom right).

Unlike primitive cells, the number of desmin-positive round rhabdomyoblasts equalled or exceeded the number of vimentin-positive cells. In many cases staining for desmin was stronger than that for vimentin. In case 8 (group M) only a few round rhabdomyoblasts, that were not found in the sections stained for intermediate filaments were present in the section stained with hematoxylin–eosin. This tumor resembled those of group P, i.e., the tumor cells were vimentin-positive and desmin-negative.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>Site</th>
<th>Growth Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6/F</td>
<td>Maxillary sinus</td>
<td>Botryoid/solid</td>
</tr>
<tr>
<td>2</td>
<td>17/M</td>
<td>Pelvis</td>
<td>Alveolar</td>
</tr>
<tr>
<td>3</td>
<td>24/M</td>
<td>Thigh</td>
<td>Alveolar</td>
</tr>
<tr>
<td>4</td>
<td>0.25/M</td>
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<tr>
<td>Group M</td>
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<td></td>
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<td>5</td>
<td>2/M</td>
<td>Retropertioneum</td>
<td>Solid</td>
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<tr>
<td>6</td>
<td>15/F</td>
<td>Tongil</td>
<td>Solid</td>
</tr>
<tr>
<td>7</td>
<td>15/F</td>
<td>Calf</td>
<td>Alveolar</td>
</tr>
<tr>
<td>8</td>
<td>5/M</td>
<td>Rhinopharynx</td>
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</tr>
<tr>
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<td>2/M</td>
<td>Pyriform area</td>
<td>Solid</td>
</tr>
<tr>
<td>10</td>
<td>8/F</td>
<td>Pharynx</td>
<td>Solid</td>
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<tr>
<td>11</td>
<td>5/F</td>
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<td>12</td>
<td>5/M</td>
<td>Paratesticular</td>
<td>Solid/Alveolar/botryoid</td>
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<td>13</td>
<td>2/F</td>
<td>Vagina</td>
<td>Solid/botryoid</td>
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<td>8/F</td>
<td>Oropharynx</td>
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<tr>
<td>16</td>
<td>7/M</td>
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<tr>
<td>Group W</td>
<td></td>
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<tr>
<td>17</td>
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<td>Vagina</td>
<td>Botryoid</td>
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<tr>
<td>19</td>
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</tr>
<tr>
<td>21</td>
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<td>Vagina</td>
<td>Botryoid</td>
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Stoop cells. These cells, present in considerable numbers only in group W, showed a staining pattern similar to that of round rhabdomyoblasts. Thus, they revealed both vimentin and desmin positivity, often with clear-cut cross-striations, but desmin positivity was more prominent, bottom right (fig. 1).

DISCUSSION

In the present study 21 cases in which a morphologic diagnosis of rhabdomyosarcoma had been made were evaluated. In all cases the mesenchymal nature of the tumor was confirmed by positivity for the mesenchymal intermediate filament vimentin. In all but four cases the myosarcomatous character was confirmed by positivity for the muscle-specific intermediate filament desmin. In five specimens from three tumors in group P, no desmin positivity could be detected. Two of these tumors were classic alveolar sarcomas of the extremities, and one was a botryoid sarcoma of the maxillary sinus. The remaining desmin-negative tumor was composed largely of primitive tumor cells but was classified as group M because of a few cells resembling round rhabdomyoblasts. Retrospectively, however, this tumor would be more properly classified as group P.

The failure to demonstrate desmin in these four cases is unlikely to have been due entirely to inadequate fixation, because normal skeletal muscle present in the same sections stained. Moreover, vimentin staining was adequate, although vimentin is reportedly more sensitive to fixation procedures than is desmin,20 and in positive specimens staining for desmin was always stronger than that for vimentin in
TABLE 2. Staining Results for Vimentin and Desmin in Relation to Cell Type

<table>
<thead>
<tr>
<th>Patient</th>
<th>Material</th>
<th>Primitive Cells</th>
<th>Round Rhabdomyoblasts</th>
<th>Strap Cells</th>
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<tr>
<td></td>
<td></td>
<td>Vimentin</td>
<td>Desmin</td>
<td>Vimentin</td>
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<tr>
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</tr>
<tr>
<td>6</td>
<td>R(2 × 1) Pa</td>
<td>±</td>
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<tr>
<td>21</td>
<td>P, Pa</td>
<td>+</td>
<td>+</td>
<td>+ +</td>
</tr>
</tbody>
</table>

**Abbreviations:** P, primary tumor; R, recurrence; B, both primary tumor and recurrence; Pa, paraffin; F, frozen material; ±, scattered positive cells; +, many, but not all, cells positive; + +, virtually all cells positive.

* No round rhabdomyoblasts present in material stained for intermediate filaments (see text).
FIGURE 1. Top, left and right, case 2, group P. Staining of primitive tumor cells for vimentin (left) and absence of staining for desmin (right). (× 350.) Bottom, left and right, case 17, group W. Strongly positive primitive cells and weakly positive round rhabdomyoblasts and strap cells in section stained for vimentin (left) and reverse staining pattern for desmin (right). (×350; inset, ×560.)
tumors. The observations regarding desmin are not surprising in view of the appearance of desmin only later in normal chick myogenesis. As for differentiated tumor cells, somewhat conflicting results have been obtained with respect to the presence of vimentin. Thus, Card and Lazarides and Granger and Lazarides found vimentin throughout all stages of muscle development, with a distribution similar to that of desmin in the later stages, whereas Bennett et al. were not able to demonstrate 58-kilodalton subunits (vimentin) in the later stages when 55-kilodalton subunits (desmin) were abundantly present. The two groups agreed, however, as to the exclusive presence of vimentin in primitive mesenchymal cells, which appear to be capable of muscle differentiation.

It is therefore tempting to assume that primitive mesenchymal tumors, such as those in group P of the present study, represent tumors with a differentiation block at a very early stage (fig. 2). A definitive myogenic lineage could be proved only by sensitive methods but seems most likely in view of the rarity of other types of soft tissue sarcomas in children. The tumors in groups M and W, in addition, contained cells arrested at a further stage of differentiation; some of these cells were morphologically recognizable as myogenic cells (round rhabdomyoblasts and strap cells), whereas others appeared myogenic only by their desmin positivity. The latter group of cells may be defined as 'committed' to myogenic differentiation and may have played an essential role in the further 'differentiation' of the rhabdomyosarcomas that was observed following chemotherapy. Briefly, in group P no morphologic changes other than necrosis were observed, whereas in groups M and W chemotherapeutic treatment resulted in increased numbers of round rhabdomyoblasts and strap cells, with the cytologic characteristics of these cells becoming more distinct (fig. 2). These effects may have been due in part to selective destruction of primitive tumor cells, but it seems more likely that the committed cells come to further development under polychemotherapy, either as a primary or a secondary effect. It might well be that in tumorigenesis the progression from primitive, desmin-negative cells, to committed, desmin-positive and further differentiated cells is accompanied by a decrease in susceptibility to the toxic effects of chemotherapy and an increase in potential to further differentiation (fig. 2; see also reference 26).

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