Alveolar Rhabdomyosarcoma

Demonstration of the Muscle Type of Intermediate Filament Protein, Desmin, as a Diagnostic Aid

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Three cases of soft-tissue sarcomas with the characteristic histologic features of alveolar rhabdomyosarcoma, but lacking cytoplasmic cross-striations, were studied ultrastructurally and immunohistochemically to confirm the diagnosis and evaluate the histogenesis. The results showed that it was not possible to judge the skeletal muscle derivation of the cells at the ultrastructural level. However, immunohistochemically, the results of every case were positive for desmin—the muscle type of the intermediate filament protein. The results suggest that demonstration of desmin may be a helpful adjunct tool in the diagnosis of poorly differentiated alveolar rhabdomyosarcomas. (Am J Pathol 1982, 108:246-251)

ALVEOLAR Rhabdomyosarcoma was introduced by Riopelle and Thieriault and by Enterline and Horn as a round cell sarcoma with peculiar alveolar-like arrangement of the tumor cells between the fibrous septa. Larger series have since shown that only a minority of neoplasms with such histologic features show cross-striations in the cells, and thus the ascertainment of the skeletal muscle nature of these tumors has remained difficult. In the majority of the cases, the same problem is also obvious at the ultrastructural level. Therefore, it may be asked whether all these neoplasms are truly of skeletal muscle cell origin. In this report we demonstrate the presence of the desmin type of cytoskeletal intermediate filaments in 3 cases of alveolar rhabdomyosarcoma. The presence of these filaments reflects the muscle cell derivation of these tumors and demonstrates the usefulness of anti-desmin antibodies as a tool in the diagnosis of poorly differentiated sarcomas.

Materials and Methods

Three cases of alveolar rhabdomyosarcoma were studied immunohistochemically and by means of light and electron microscopy. The following are short case reports.

Case 1: An 11-year-old girl had a swelling at the base of the nose, and a subcutaneous tumor 2 cm in diameter was extirpated. Two local recurrences developed within a year. One of the recurrences was available for this study.

Case 2: A 23-year-old woman had noticed a tumor 3 cm in diameter on her left foot. Simultaneously, she found an inguinal tumor, which was interpreted histologically as a metastasis. The tumor from the foot was extirpated and studied in detail.

Case 3: A 17-year-old girl had noticed a tumor that had grown rapidly in the vulva. The tumor, which was 6 cm in diameter, was removed. The patient died 8 months later because of widespread metastases.

Paraffin blocks were available for all of the tumors. Hematoxylin and eosin (H&E), phosphotungstic acid hematoxylin (PTAH), and periodic acid–Schiff (PAS) staining with and without prior diastase treatment was used in all cases.

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Specimens for electron-microscopic examination were taken from formalin-fixed tissue in 2 cases and from a dewaxed piece of a paraffin block in 1 case. Small tissue pieces were postfixed in 0.5% osmium tetroxide in 0.1 M phosphate buffer, at pH 7.2; dehydrated in acetone, and embedded in Epon 812. Five to 10 blocks were made in each case, and a cellular area was chosen for thin sections with the use of semithin sections of 1 µ stained with toluidine blue. Thin sections were poststained with lead citrate and uranyl acetate and examined in a Hitachi HS-7S electron microscope at an acceleration voltage of 50 kv. One block and 3–5 grids of each case were examined.

Desmin was purified from chicken gizzards according to Small and Sobieszek. Antiserum was raised in rabbits and purified on a desmin-Sepharose CL4B column. The purified antiserum stained typically frozen sections of skeletal muscle and cultured rhabdomyosarcoma cells, but not fibroblasts or fibrosarcoma cells, fibrosarcoma, or adipose tissue sections viewed by immunofluorescence microscopy. On the other hand, tumors consisting of vimentin-positive cells, like epithelioid sarcoma, were negative for desmin. Similarly, no cross-reactivity of the desmin antibodies with keratin, neurofilament proteins, or glial fibrillary acidic protein could be found.

Immunohistochemical staining for desmin was performed as follows. The 3–5 µ-thick paraffin sections were deparaffinized and treated with 0.4% pepsin in 0.01 N HCl for 2 hours. Endogenous peroxidase was blocked by incubation of the slides for 30 minutes in methanol containing 0.2% H2O2. After being washed, the slides were incubated with rabbit anti-desmin antibodies (25 µg/ml). The slides then were exposed to a biotinylated anti-rabbit immunoglobulin antiserum, (dilution 1:500), avidin (dilution 1:1000), and biotinylated horseradish peroxidase complex. The reagents were purchased from Vector Laboratories (Vectastain, Burlingame, Calif). We developed the peroxidase reaction by in-

Figure 1 — Alveolar rhabdomyosarcoma shows round, medium-sized, relatively uniform cells with formation of alveolar or glandlike structures between the connective tissue septa. No rhabdomyoblastic features are evident light-microscopically. (H&E, × 400) (With a photographic reduction of 2%)
cubating the slides in 0.01% H$_2$O$_2$ and 0.05% 3'-3'-diaminobenzidine tetrahydrochloride (Fluka AG, Buchs, Switzerland) for 10 minutes in the dark. The slides were counterstained for nuclei with Meyer's hematoxylin for 1 minute, rinsed, and mounted in Aquamount. The results of control stainings, made with preimmune serum or serum run through the desmin-Sepharose 4B column, were negative.

**Results**

**Light Microscopy**

The tumors were closely similar at the light microscopic level, showing rounded, medium-sized uniform cells with diffusely hyperchromatic nuclei, usually containing inconspicuous nucleoli and having sparse, but occasionally more copious, eosinophilic cytoplasm. The cell cohesion often was poor, and the clefts had an alveolar or glandlike appearance (Figure 1). At some areas, the tumors had a solid pattern (Figure 2). Thick fibrous septa containing blood vessels irregularly lobulated the tumor tissue. Mitoses were numerous; there were three to four in the high-power field in the cellular areas. In 2 cases, the tumor infiltrated and destroyed adjacent striated muscle tissue. Many cells showed cytoplasmic diastase-labile PAS-positivity (ie, glycogen). Cytoplasmic cross-striations after PTAH staining were not found in any of the cases.

**Electron Microscopy (EM)**

The tumor cells were uniform in appearance, having rounded nuclei and evenly distributed chromatin. The cytoplasm was sparse and contained only few organelles, such as short strands of rough endoplasmic reticulum, and few mitochondria. Clusters of polyribosomes were often numerous. In 1 case, some neoplastic cells facing the collagenous stroma were endowed with a well-defined basal lamina (external lamina) (Figure 3). No specific signs suggesting rhabdomyosarcoma, such as myofilament bundles or sarcomeres, were seen in the cells of any of the tumors.

**Immunohistochemical Findings**

All 3 cases showed cytoplasmic desmin positivity, especially in the cells lining the alveolar clefts. Some neoplastic giant cells with abundant cytoplasmic desmin staining were seen in 1 case (Figure 4). In solid

![Figure 2](https://example.com/figure2.jpg) - Alveolar rhabdomyosarcoma may show a solid pattern of growth at places. (H&E, x 400)
areas, the tumor cells stained positively for desmin only occasionally. The entrapped striated muscle cells were also positive for desmin (Figure 5). The results in fibrous septa were completely negative, and the background staining was minimal.

Discussion

The results and previous reports show that muscle derivation of poorly differentiated alveolar rhabdomyosarcomas is difficult to demonstrate by light and even by electron microscopy. However, the EM sampling was limited and no large cells (Figure 4) were found in the EM sections.

In this study we show that desmin, the major subunit protein of the muscle type of intermediate filaments can be demonstrated immunohistochemically in paraffin-embedded tissues and used as a marker of muscle differentiation. However, desmin is not specific for skeletal muscle; it is also seen in some smooth muscle cells, although not in all. None of the histologic features of our cases, however, suggested leiomyosarcoma.

In this study, all 3 cases of alveolar rhabdomyosarcoma showed positive results for desmin. Because only a portion of the cells showed positive staining, it is understandable that ultrastructural methods showed no specific features. It seems likely that immunohistologic techniques are superior to ultrastructural methods in recognizing poorly differentiated cells of skeletal muscle derivation that may lack typical ultrastructural organization. Moreover, immunohistologic techniques more easily enable a wide and rapid sampling of specimens. Recently, Gabbiani et al have shown desmin positivity in rhabdomyosarcoma by the immunofluorescence technique, and Virtanen et al showed desmin positivity in cultured rhabdomyosarcoma cells. A precaution must be taken into account, however, when one is using immunohistochemical methods. One must ascertain that the cells evaluated for the diagnosis truly are neoplastic cells, because, for example, entrapped muscle cells also stain positively for desmin. The counterstain with hematoxylin is a valuable aid in the localization of positive staining in the tumor tissue.

Recently, antibodies against different types of cy-

Figure 3—At the ultrastructural level, the tumor cells are poorly differentiated with sparse organelles. No structures specific for a skeletal muscle cell can be discerned. This cell, facing a connective tissue septum, is endowed by a well-defined basal lamina (arrow). C = collagen. (× 23,000) (With a photographic reduction of 2%)

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toskeletal intermediate filament (IMF) proteins, have been used successfully in differential diagnosis of epithelial and mesenchymal liver tumors\(^6\) and in the detection of glial cells in amniotic fluid in cases of anencephaly.\(^{17,18}\) In a recent survey, Gabbiani et al\(^9\) even proposed IMF antibodies as a general aid in distinguishing poorly differentiated tumors, because it appeared that tumor cells retained their specific type of IMF proteins.\(^{10}\)

Myoglobin also has been shown to be a suitable marker for skeletal muscle differentiation\(^{19,20}\) with the use of immunoenzymatic technique. Corson and Pinkus\(^20\) showed myoglobin-positive neoplastic cells in 5 of 7 cases of alveolar rhabdomyosarcoma. Unlike desmin, myoglobin is specific for skeletal muscle and does not occur in smooth muscle cells.\(^{18}\)

It has been suggested recently that methods of immunofluorescence would be superior to methods based on immunoenzymatic techniques.\(^{15}\) However, our results clearly show the usefulness of the peroxidase method in combination with conventional histologic staining, which enables the use of all histologic criteria, in addition to the immunohistochemical findings. We also found that the peroxidase technique used with untreated sections gave rather poor results, which could be markedly improved by protease treatment of the deparafined sections, as has been suggested previously with trypsin\(^{21,22}\) and with pepsin.\(^{23}\) This method also appears to make it possible for one to carry out retrospective surveys using routine paraffin-embedded material.

**References**


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