Differentiated Embryonal Rhabdomyosarcoma in a Cow

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ABSTRACT. An embryonal rhabdomyosarcoma was found in the pleura of a 2-year-old Holstein cow after first delivery. The most predominant cells in the tumor were relatively small in size, but considerable numbers of more differentiated cells of larger sizes mingled with the small cells. The most differentiated cells were characterized by multinucleation, abundant cytoplasm containing cross-striated fibrils, intense immunoreactivity for desmin, and weak or negative reactivity for vimentin. Such cells, lacking mitotic activity and displaying weak or no reactivity for proliferating cell nuclear antigen, were considered to be malignant counterparts of myotubes or muscle fibers. This neoplasm seems to follow normal skeletal muscle embryogenesis, and to be capable of differentiation into the final stage of muscle development.—KEY WORDS: bovine, immunohistochemistry, rhabdomyosarcoma.

In humans and animals, there are three major categories of rhabdomyosarcoma: pleomorphic, embryonal, and alveolar [15]. These three forms have been investigated immunohistochemically in dogs [17, 19, 23], while desmin and myoglobin immunohistochemistry was carried out on a highly undifferentiated rhabdomyosarcoma of alveolar type in a 7-year-old cow [18]. Human embryonal rhabdomyosarcomas have been subdivided into differentiated and poorly differentiated types [2]. Here we describe the histological and immunohistochemical features of a bovine rhabdomyosarcoma with giant cells having numerous nuclei and distinct cross-striations.

A 2-year-old Holstein cow was anorexic and depressed one month after first delivery, and had diarrhea 11 days later. Because the clinical condition was unchanged, the cow was euthanatized 2 months after delivery. At necropsy, the pericardial pleura, which had a tumor mass (10 cm in diameter) on its surface, was covered with large amounts of blood clot. The tumor was well encapsulated, and was partially adherent to the pulmonary pleura. The cut surface was grayish white in color, homogeneous and elastic in consistency, with a widespread area of central necrosis. Neoplastic lesions were absent on the surface of the serous pericardium. The lungs were somewhat edematous, and subcutaneous edema was located in the intermandibular space and the ventral aspects of the neck and trunk.

Tissues were fixed in 10% buffered formalin, embedded in paraffin wax, cut at 4 µm, and stained with hematoxylin and eosin (HE), phosphotungstic acid-hematoxylin (PTAH), periodic acid-Schiff (PAS), PAS with diastase predigestion (DPAS), Schmorl’s method for lipofuscin, Ziel-Nielsen, Fontana-Masson, and Prussian blue stains. Additionally, the avidin-biotin-peroxidase complex immunoperoxidase technique (ABC-IP) was applied to paraffin wax-embedded sections. The following were used as primary antibodies: rabbit polyclonal antibody to myoglobin (Nichirei Corporation, Tokyo, Japan), and mouse monoclonal antibodies to desmin (Bio-Science Products, Emmenbrücke, Switzerland), alpha-smooth muscle actin (SMA), vimentin (Dako Corporation, Carpinteria, CA, U.S.A.), and proliferating cell nuclear antigen (PCNA) (BioGenex Laboratories, San Ramon, CA, U.S.A.). An immunoperoxidase labeling kit (BioGenex Laboratories) was used in the subsequent processes.

Histologically, the tumor was composed of densely packed cells, which were extremely variable in size. Relatively small cells, however, predominated in number, and larger cells showed a tendency of forming groups (Fig. 1). In parts, hemosiderin or lipofuscin was deposited in some neoplastic cells, and erythrophagia by neoplastic cells were rarely seen. Granular PAS-positive, DPAS-negative material was not observed.

The small cells were round to spindle-shaped, and contained round to spindle-shaped nuclei, many of which showed irregular contours. The nuclei were inconspicuous or modest in size, and the chromatin was moderately clumped. The cells had small to moderate amounts of cytoplasm, but showed a transition into larger cells with abundant cytoplasm. Mitotic figures were frequently seen.

The larger cells varied in size from cells with single nuclei to giant cells with large numbers of nuclei, and were characterized by abundant cytoplasm containing eosinophilic fibrils, which frequently showed cross-striations (Figs. 2, 3). Many of the nuclei were similar in size and shape to those of small cells, but the chromatin tended to be more finely clumped. The giant cells varied in shape from rounded, ovoid, polyhedral, spindle-shaped, and ribbon-shaped to circular (Fig. 3) and cylindrical cells (Fig. 4), and their nuclei were arranged in clusters, in heaps, in chains, in rows, in rings, or disorderly. Giant cells with many nuclei were liable to be degenerative with dissolution of myofibrils. Mitoses were few in mononucleated cells, and absent in multinucleated cells.

There were thick walls of dense fibrous tissue between the tumour and the pulmonary parenchyma, but a few neoplastic cells were present in the alveoli and the lumens of blood vessels and lymphatics.

Immunohistochemically, almost all larger cells were intensely positive for desmin (Fig. 5). Many small cells were weakly positive for desmin though some were negative.
Myoglobin was expressed in several giant cells alone, and staining for SMA was negative in all neoplastic cells. Although small cells stained positively for vimentin, many larger cells stained weakly or negatively (Fig. 6). Many small cells were strongly reactive for PCNA, whereas larger cells were weakly positive or negative (Fig. 7). Intra-alveolar or intravascular desmin-positive neoplastic cells could be confirmed (Fig. 8).

Myoglobin synthesis occurs rather late in striated muscle differentiation; both desmin and actin tend, therefore, to be rather more sensitive in detecting poorly differentiated rhabdomyosarcomas in human beings [3]. In our case, the majority of the neoplastic cells were positively labeled by the anti-desmin antibody, but myoglobin-positive cells were relatively few. Desmin is an excellent marker to detect myoblastic differentiation [6], and negative reactivity for SMA is valuable in excluding the possibility of leiomyosarcoma [14] in a bovine malignant muscle tumour expressing desmin.

Embryonal rhabdomyosarcoma arises from undifferentiated mesenchyme of young children, and consists of sheets of primitive round cells and differentiating rhabdomyoblasts admixed in various proportions [22]. By contrast, pleomorphic rhabdomyosarcoma is an extremely rare and highly malignant neoplasm, usually occurs in voluntary muscles of adults, and is composed almost
exclusively of large pleomorphic rhabdomyoblasts [22]. Embryonal rhabdomyosarcoma commonly affects young dogs [1, 16, 23]. In the present neoplasm arising in the pleura of a 2-year-old cow, the most predominant cells were relatively small in size, but there were considerable numbers of large, well-differentiated cells showing cross-striations or strong reactivity for desmin, and hence a diagnosis of differentiated embryonal rhabdomyosarcoma was made [2].

In human pleomorphic rhabdomyosarcomas, cross-striations are usually not identified by light microscopy [11, 13]. In the tumor described here, multinucleated cells, characterized by weak or negative reactivity for PCNA and lack of mitoses, were considered to be resting or terminally differentiated cells showing cross-striations or strong reactivity for desmin, and hence a diagnosis of differentiated embryonal rhabdomyosarcoma was made [2].

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In the tumor described here, multinucleated cells, characterized by weak or negative reactivity for PCNA and lack of mitoses, were considered to be resting or terminally differentiated cells [10] and to be malignant counterparts of myotubes or muscle fibers with multiple nuclei [9]. As in normal skeletal myogenesis or in muscle regeneration, multinucleation may have been caused mainly by cytoplasmic fusion of mononuclear cells [5, 8]. In contrast, small cells, many of which showed intense positivity for PCNA, were thought to correspond to myoblasts in the active phases of the cell cycle [10]. This view is supported by desmin and vimentin staining. Vimentin is known to be present in the early stages of normal skeletal muscle development, and later in the myogenesis, vimentin is partially or completely replaced by desmin [4, 21]. Similarly, in our case, the greatest intensity of vimentin was visualized in small immature cells, whereas that of desmin was in large well-differentiated cells. Although human rhabdomyosarcomas seemed to follow normal skeletal myogenesis without completing the final step [20], the present tumor had highly differentiated cells mimicking normal or regenerating muscle fibers [12]. Spindle cell rhabdomyosarcoma, which is a recently recognized variant of embryonal rhabdomyosarcoma in human beings, is composed of elongated spindle cells with cross-striations [7]. Such cells were thought to be highly differentiated and to be at the late myotube stage of myogenesis, but they usually contained single nuclei unlike normal myotubular
cells [22]. In our case, neoplastic cells having both of the multiple nuclei arranged in chains and the extremely elongated cytoplasm with cross-striations were considered to be cells recapitulating the myotube stage of myogenesis.

REFERENCES