Thymic Carcinoma of the Thymic Hormone Secretory Type in a Cow
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(Received 29 October 1998/Accepted 6 January 1999)

ABSTRACT. An 8-year-old Holstein cow had tumor nodules and enlarged lymph nodes in the mediastinum, and metastatic tumor masses in the pelvic cavity. The neoplastic cells were characterized by squamous features and intracytoplasmic vacuoles carrying microvilli, some of which contained periodic acid Schiff-positive globular cores, but tubular structures or goblet cells were absent. Many neoplastic cells stained positively for keratin, and occasional cells were positive for thymosin. The presence of secretory granules in the cytoplasm was confirmed by electron microscopy. This neoplasm was considered to be of thymic hormone-secreting epithelial cell origin.—KEY WORDS: bovine, thymic carcinoma, thymosin.


Thymic carcinoma is rare in domestic animals, but has been reported in dogs [1, 2], cats [3, 6], a horse [21], and a cow [13]. Although squamous cell carcinoma or lymphoepithelioma-like carcinoma predominates in human thymic carcinomas, mucoepidermoid carcinoma [18], adenosquamous carcinoma [17] and adenocarcinoma [4] have been observed. There were keratinization and vesicle formation in a bovine thymic carcinoma [13] and glandular differentiation in a canine thymic carcinoma [1].

Thymic hormones have been demonstrated in the epithelial cells of the normal human thymus [7, 14] and human thymoma cells [11]. Such hormones are considered to be secreted by thymic epithelial cells with intracytoplasmic vacuoles [10, 20]. Thymosin was detected in the epithelial cells of the calf thymus [19]. Here we describe a bovine thymic carcinoma, whose outstanding features were intracytoplasmic vacuoles and thymosin production.

An 8-year-old Holstein cow showed diarrhea and anorexia. Because the condition did not improve and abdominal tumor masses were detected on examination per rectum, the animal was killed humanely 13 days after onset of clinical signs.

At necropsy, the mediastinum was filled with nodules of varied sizes and enlarged lymph nodes. On cut section, these tissues were grayish white with scattering foci of necrosis or hemorrhage. There were several well-demarcated subpleural tumor metastases, 2 to 3 cm in diameter. The pelvic cavity contained many tumor masses and nodules.

Tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin wax, and routinely processed for histological examination. Sections were stained with hematoxylin and eosin (HE), periodic acid/Schiff (PAS), and Alcian blue (pH 2.5). Immunohistochemistry was applied to paraffin wax sections. The samples were incubated with rabbit polyclonal antibodies to keratin (BioGenex Laboratories, U.S.A.) and thymosin (Biogenesis, U.K.), and an immunoperoxidase staining system (BioGenex Laboratories) was used in the subsequent processes. For immunohistochemical comparison, paraffin wax sections of the thymus from a healthy calf were treated for thymosin staining. For electron microscopy, small blocks taken from formalin-fixed tissues were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

Histologically, the mediastinal nodules had many foci of necrosis, and were encapsulated by dense collagenous tissue, but neoplastic cells were seen in surrounding adipose tissue and within lymphatic vessels. The neoplastic tissue consisted of solid clumps or branching lobules of large cells, with stromal fibrosis. Some tumor cell nests were encircled by many lymphocytes (Fig. 1). The neoplastic cells, which resembled squamous cells, were polyhedral and had large round or ovoid vesicular nuclei, occasionally with irregular or binuclear profiles. The nucleoli were moderately prominent. The cytoplasm was eosinophilic, frequently with vacuoles of varying sizes, some of which were lined by microvilli or contained eosinophilic hyaline globules (Figs. 2, 3). Many vacuoles contained Alcian blue-positive material, but hyaline globules did not stain with Alcian blue and were PAS-positive and diastase resistant. Mitotic figures were abundant, and in places, intercellular bridges could be identified. The other neoplastic lesions had similar histological features, but the lesion in the pelvic cavity had extensive areas of necrosis, and the neoplastic cells became frequently non-cohesive, being more pleomorphic and more atypical than those in the mediastinal nodules. This lesion was not accompanied by infiltration of lymphocytes. The thymic tissue from a healthy calf appeared normal, and had epithelial-recticular cells with cytoplasmic vacuoles in some thymic corpuscles.

Immunohistochemically, many neoplastic cells were positive for keratin. The neoplastic cells occasionally showed positive cytoplasmic or vacuum membrane staining for thymosin (Fig. 4). Some hyaline globules were also positive for thymosin. In the thymus of a calf, the epithelial-recticular cells frequently showed cytoplasmic staining for thymosin.
Fig. 1. Vacuoles are sparsely distributed in islands of neoplastic cells, separated by bands of lymphocytes. HE stain. × 100.

Fig. 2. In this field, there are relatively many vacuoles, some of which contain globules (arrows). HE stain. × 200.

Fig. 3. A neoplastic cell has a vacuole, in which a central core and microvilli are evidently seen (arrow). Binuclear profiles are detectable (arrowheads). HE stain. × 400.
Ultrastructurally, frequent cells had intracytoplasmic vacuoles with or without electron-dense globules, and microvilli projected into the lumina (Fig. 5). The rough endoplasmic reticulum (RER) was slightly to moderately developed, sometimes showing focal dilatation with moderately dense intracisternal contents (Fig. 6). There were a few to several dense secretory granules and considerable amounts of intermediate filaments, but sheaves of tonofilaments were few. The neoplastic cells had a few to numerous cytoplasmic processes or microvilli on their surfaces, and desmosomes were seen between adjoining cells, but it was very rare to find desmosome-tonofilaments complexes. Electron-dense material similar to that within vacuoles was present extracellularly (Fig. 6).

Although the pelvic cavity was filled with tumor growths, the neoplasm described here was readily distinguishable from a squamous cell carcinoma of abdominal organ origin. The present neoplasm had no relation to certain abdominal organs, and the most striking histological feature was the formation of intracytoplasmic vacuoles, which was absent in ordinary squamous cell carcinomas. The neoplastic lesion in the pelvic cavity, consisting of more anaplastic cells than the mediastinal lesion, could be accepted as a metastasis.

Intracytoplasmic crypts or lumina with microvilli have been seen in various types of human tumors, but the majority were adenocarcinomas with glandular differentiation [8, 9]. In human beings, normal thymic epithelial cells in the medulla had intracytoplasmic vacuoles suggesting a secretory capacity [20] and small vesicles containing small dense globules or membranous materials [10]. Savino and Dardenne [14] observed that thymic hormones such as thymosin and thymulin were present exclusively in the epithelial cells of the human thymus, and suggested that the production of different hormones in the thymus was accomplished by the same epithelial cells. In our study, the neoplastic cells had intracytoplasmic vacuoles and evidence of squamous differentiation, and expressed thymosin positivity, which has been demonstrated in bovine thymic epithelium [19]. On the basis of such findings, the tumor was thought to have arisen from thymic epithelium. In rat and murine thymuses, thymulin-positive material was demonstrated within vacuoles of epithelial cells [15, 16]. Thymic carcinomas were divided into several histological types in human beings, but not in dogs [5]. An adenocarcinoma variant of thymic carcinoma, however, was reported in a dog [11], and feline [6] and equine [21] squamous cell carcinomas of the thymus were characterized, respectively, by keratinization and structures resembling Hassall’s corpuscles. Human thymic carcinomas with squamous differentiation may show keratinization [18] or glandular differentiation [12]. Although a bovine thymic carcinoma revealed both single cell keratinization and vesicle formation, almost all of the tumor cells had eosinophilic granules resembling keratohyaline granules and a few tumor cells contained round vesicles [13]. This neoplasm may be categorized roughly into the keratinized type. In contrast, the present neoplasm, which was characterized by intracytoplasmic vacuoles and was devoid of keratinization and keratohyaline granules, may be closely related to hormone-secreting epithelial cells, but was apparently different from adenosquamous carcinoma and mucoepidermoid carcinoma, in which tubular structures or mucin-producing cells were indispensable elements [17].

REFERENCES


Fig. 4. Cytoplasmic vacuoles outlined in black represent a positive reaction for thymosin, and intraluminal globules are faintly positive. 4,000.
Fig. 5. This neoplastic cell possesses a cytoplasmic vacuole lined by microvilli, which contains not only a large electron-dense globule but also several smaller ones, adherent to the luminal membrane (arrows). Secretory granules are scanty (arrowheads). 6,000.
Fig. 6. There are secretory granules with electron-dense cores in the cytoplasm of neoplastic cells, and electron-dense material is visible extracellularly (arrows). Arrowhead indicates an expanded cisterna of RER. 4,500.