A Spontaneous Teratoma in an Axolotl (*Siredon mexicanum*)

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SUMMARY

A spontaneous tumor originated in the dorsal muscles of the proximal portion of the tail of a 2.5-year-old axolotl (*Siredon mexicanum*). Histologically, the tumor consisted of tissues derived from ectoderm, mesoderm, and endoderm. Its most significant cells were epithelial, partly organized in the form of gland-like structures. The neoplastic glandular epithelium was arranged mostly in solid sheets or cords resting on a thin basement membrane; this tissue did not resemble any of the well-developed or differentiated epithelium or glandular tissue known to occur in the axolotl. The cells contained scanty cytoplasm with abundant basophilic material. Their nuclei were large and hyperchromatic and had one or two prominent nucleoli. Only these cells underwent mitosis, which was extensive in some areas. A second common tissue most likely resulted from differentiation and gradual degeneration of the epithelial tumor tissue, producing rounded pieces of compact tissues with a comparatively small number of nuclei and gradually increasing amounts of intercellular material. The nuclei became pyknotic and fewer in number. The tumor also contained loose, compact, and embryonic connective tissues. In some areas, there were neuroepithelial structures, isolated striated muscle was found. In some areas there were neuroepithelial structures and abnormal cartilage. There were few blood vessels, many blood sinuses, and many blood cells in various stages of development.

This tumor was evidently a spontaneous teratoma.

INTRODUCTION

Amphibian and human neoplasms show remarkable similarities in cellular structure (4). Almost all human tumors have their analogs in other animals, but most studies have dealt with blooded vertebrates. fishes, mostly because of the assumption that tumors in cold-blooded vertebrates are rare and hence difficult to obtain for study (6). Lucké and Schlumberger (7) showed otherwise in fishes and amphibians. Even so, tumors appear to be less frequent in urodeles than in anurans (1).

The Mexican axolotl (*Siredon mexicanum*) is the neotenic larval form of a salamander, which nevertheless becomes capable of reproduction (2). Axolotls have developed several types of tumors, melanoma (5, 10, 11, 13, 14, 16), adenocarcinoma (13), neuroepithelioma (3), and possibly epithelioma (12), but such spontaneous tumors are rare. According to Stevens (15), the number of species in which teratomas have been observed is small. Teratomas have never been noted in dogs, rats, rabbits, or amphibians.

MATERIALS AND METHODS

A spontaneous tumor was discovered of the left proximal portion of the tail of a 2.5-year-old axolotl. The lumbosacral region of the animal, together with the proximal portion of the tail, was fixed in toto in a modification of Zenker's fixative (A. L. Roque, manuscript in preparation). Superficial incisions were made in order to facilitate penetration of the fixative. A couple of hours later, when the tissues were hardened, the tumor and the surrounding tissues were cut transversely into four pieces for gross examination.

After each piece of tumor had been photographed, it was embedded in paraffin in toto. Sections at 6 μ were made from both surfaces of each block and were stained with hematoxylin and eosin for general histologic study and photomicrography. For demonstrating connective tissue, a trichrome stain (9) and the Puchtler phosphotungstic acid hematoxylin stain (8) were used. Periodic acid-Schiff (PAS) and mucicarmine were employed for demonstrating glycogen and other polysaccharides. A modification of the May-Grünwald-Giemsa technic was used for demonstrating basophilic and acidophilic cytoplasmic materials. Photomicrographs were made at X 15, 40, 100, 200, and 610. Special attention was given to the preparation of one compound photograph consisting of many separate photomicrographs under very low magnification (X 15), in order to provide a general picture of an almost complete cross section of the tumor.

RESULTS

While the animal was still alive, the tumor measured 19.7 x 19.8 x 8.2 mm (Fig. 1). It bulged under the skin, which was compressed by it. The tumor extended deep into the subcutaneous tissue and penetrated some distance into the dorsal muscles, which were partially destroyed, compressed, and displaced (Figs. 2, 3). It had a loculated appearance, being composed of cavities separated by thin septa of connective tissue. Some of these cavities were cystic and contained a mucoid material. Others contained a solid material of epithelial appearance.

Histologic examination showed that the tumor consisted of a wide variety of tissues, foreign to the surrounding muscle (Fig. 7) and mostly atypical for a normal organism. The most important tissue was epithelium, partly organized in the form of gland-like structures (Fig. 4). These neoplastic glands were composed of cuboidal or cylindrical cells arranged in various layers. They rested on a basement membrane well demonstrated by the PAS and reticulum-trichrome stains. Some...
glands had a wide, empty lumen; in others, the lumen was filled with a mucoid material that reacted positively to mucicarmine and PAS and was visible in tumor cytoplasm. Accumulation of this material seemed to have brought about the distension and cystic degeneration of the neoplastic gland-like structures; nuclei of tumor cells were embedded in it (Fig. 28). Some of this material was still recognizable as debris from the cytoplasm of tumor cells.

The neoplastic glandular cells were arranged mostly in solid sheets or cords resting on a thin basement membrane (Figs. 5–8, 10, 11, 15). These tumor cells contained scanty cytoplasm with abundant basophilic material; their nuclei were large and hyperchromatic, and had one or two prominent nucleoli. An occasional giant cell, probably triploid (Fig. 16), was observed. Only these glandular neoplastic cells underwent mitosis, which was extensive in some areas (Figs. 16–21). An apparent minor modification was found in sheets of epithelium, consisting mostly of one layer of cylindrical cells (Figs. 7–9, 12). This epithelium sometimes formed the walls of large cavities (Figs. 7–9) or penetrated between other compact tissues (Fig. 12).

Most likely it was differentiation and gradual degeneration of the glandular neoplastic tissue that resulted in the development of the second most significant tissue. This consisted of rounded pieces of compact tissue with a relatively small number of nuclei and a gradually increasing amount of intercellular material. These pieces of tissue had definite borders and were structurally similar to cartilage (Fig. 7A). The intercellular material, presumably representing the beginning of the development of this tissue, was almost completely homogeneous (Fig. 11), but the process of differentiation resulted in the formation of numerous fibers (Figs. 5, 12, 14, 15). This tissue occurred mostly in large pieces (Fig. 7) but was sometimes subdivided into many small, rounded pieces (Figs. 7, 13); mitosis was never observed. Evidently the tissue had gradually degenerated, the nuclei decreasing greatly in number and becoming pyknotic. Giant degenerate nuclei were observed in some cases (Fig. 14). Such tissue is never present in a normal axolotl.

The tumor also contained small amounts of other types of tissues (Fig. 7), particularly the connective tissue of the septa separating the lobules of the tumor. In some places, there was embryonic fibrous tissue, easily distinguished from adult connective tissue with its bands of collagen fibers (Figs. 5, 23, 24, 28), being composed of fibroblasts laying down fibers of collagen, as shown by trichrome stain. The trichrome and the Puchtler phosphotungstic acid hematoxylin stains revealed fibroblasts in the cytoplasm of these fibroblasts.

In the septa composed of connective tissue, isolated pieces of striated muscle were found (Figs. 10, 26, 28). These pieces were independent of the striated muscles surrounding the tumor, just as the loose connective tissue and the epithelium of the tumor were independent of the subcutaneous loose connective tissue and the skin epithelium in the vicinity of the tumor (Fig. 7). A few normal pigment cells (melanocytes) were also present, chiefly in connective tissue (Fig. 10).

Small pieces of abnormal cartilaginous tissue were found in some portions of the tumor. This tissue contained intercellular material typical of cartilage but completely lacked cartilaginous cells. In empty spaces where cartilaginous cells might be expected to occur, there were only blood cells (Fig. 25); some pieces of cartilage contained large blood sinuses. In other places, however, there were small clusters of epithelial cells which, because of their form, structure, and arrangement, can be regarded as neuroepithelium (Fig. 27).

Only a few (mostly small) true blood vessels were found in the tumor (Figs. 7, 9, 10, 12, 23); all had blood vessel walls (Fig. 23). More often the blood supply of the tumor was provided by large blood sinuses (Fig. 7). These sinuses characteristically lacked blood vessel walls. Some were surrounded by simple epithelium, others partly by epithelium and partly only by a thin basement membrane (Fig. 22), and still others only by a cartilaginous wall. In many places, the blood sinuses had no definite borders, and blood cells in large numbers penetrated into the degenerate differentiated tissue that predominated in the tumor (Fig. 7). It is noteworthy that large numbers of erythrocytes and leukocytes, in various stages of development, accumulated in the blood sinuses.

DISCUSSION

A single tumor, composed of tissues derived from ectoderm, mesoderm, and endoderm, was observed in one animal; no metastasis was found. It seems to have originated in skeletal muscle, but neither its gland-like epithelial tissue nor the tissue probably resulting from differentiation of that epithelial tissue resembled any of the well-developed epithelium, glandular tissues, or other tissues of the normal adult axolotl. Thus the tumor satisfies Willis' definition of a teratoma as "a true tumor or neoplasm composed of multiple tissues foreign to the part in which it arises" (17). At least one gross feature of the tumor, cystic cavities in which solid components were growing, was also characteristic of a teratoma.

REFERENCES

A Spontaneous Axolotl Teratoma


Fig. 1. Axolotl, 2.5 years old, with spontaneous tumor (T) on proximal dorsal portion of tail. Centimeter rule is included for comparison.
Fig. 2. Cross section of whole animal shown in Fig. 1, rostral to tumor; normal situation of tissues. M, muscle; S, spinal cord; V, vertebral column. Centimeter rule is included for comparison.
Fig. 3. Two cross sections of animal shown in Fig. 1. M, muscle; S, spinal cord; T, tumor; V, vertebral column. Centimeter rule is included for comparison.
Fig. 4. Tumor cross section. G-C, glandular cell tissue. In many places, a glandular tube with an empty lumen (L) was formed. \( \times 40 \).
Fig. 5. Tumor cross section. CT, loose connective tissue; DT, differentiated tissue derived from glandular tissue (GC). \( \times 40 \).
Fig. 6. Tumor cross section. This portion of the tumor consisted only of glandular cells, and its lobules were separated by septa (SE) composed of connective tissue. There was little lumen (L). \( \times 40 \).
Fig. 7. Compound photomicrograph (prepared from many separate photomicrographs) of almost complete cross section of tumor. A, differentiated tissue, resulting from transformation of glandular cells (D); B, tissue consisting of sheets of epithelium; BS, blood sinuses; BV, blood vessels; C, tissues consisting of limited numbers of glandular cells and large empty lumens; CT, loose connective tissue; E, skin epithelium; M, muscle (muscle inside the tumor, as well as normal muscle outside); MT, tumor margin. \( \times 15 \).
Fig. 8. Tumor cross section. BC, blood cells in blood sinus; DT, differentiated tissue; E, sheets of epithelium; GC, glandular cells; L, lumen. \( \times 40 \).
Fig. 9. Epithelial part of tumor. BV, blood vessel; E, sheet of epithelium; MM, mucoid material in the lumen. \( \times 100 \).
Fig. 10. Connective tissue septa (CT) between glandular lobules (GC). BV, blood vessels; M, muscle; PC, pigment cells. \( \times 200 \).
Fig. 11. Glandular (GC) and differentiated (DT) portions of tumor. \( \times 100 \).
Fig. 12. Epithelial layer (E) between differentiated portions (DT) of tumor. BV blood vessels. \( \times 100 \).
Fig. 13. Differentiated portion of tumor, consisting mostly of pycnotic cells. \( \times 200 \).
Fig. 14. Degenerate portion of tumor, with pycnotic nuclei (P) or degenerate giant nuclei (G). \( \times 200 \).
Fig. 15. Portion of tumor, with final differentiation (DT). GC, glandular cells. \( \times 200 \).
Figs. 16—21. Glandular cells of tumor. G, giant cell; Mt, mitotic figures. \( \times 610 \).
Fig. 22. Blood sinus in tumor, lined with epithelium (E) and thin basement membrane (M). BC, blood cells. \( \times 200 \).
Fig. 23. Portion of tumor, consisting of glandular cells (GC) and loose connective tissue (CT) surrounded by thin simple squamous epithelium (E). BV, large blood vessel; PC, pigment cells. \( \times 200 \).
Fig. 24. Connective tissue between tumor lobules. CTC, connective tissue cell; F, collagen fibers. \( \times 610 \).
Fig. 25. Abnormal cartilage in tumor. BC, blood cells in the spaces (S) in the intercellular substance (CR). \( \times 610 \).
Fig. 26. Striated muscle (M) in connective tissue (CT). \( \times 610 \).
Fig. 27. Neuropithelial cells (N). CT, loose connective tissue. \( \times 610 \).
Fig. 28. Portion of tumor, consisting of glandular tissue (GC) and interstitial connective tissue (CT), with striated muscle (M). L, lumen; MM, mucoid material with nuclei. \( \times 200 \).
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