Melanotic Neurofibroma in a Steer

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ABSTRACT. A melanotic neurofibroma in a steer was investigated histologically, immunohistochemically and ultrastructurally. A very large tumor mass was located in the region of the head and right cheek. The tumor tissue consisted of an admixture of cells resembling Schwann cells and spindle-shaped cells, and they frequently contained melanin granules. Neoplastic Schwann cells were positive for S100 protein, with variation in intensity of staining, but most spindled cells were S100 negative. The tumor cells displayed ultrastructural features similar to those of Schwann cells or perineurial cells. The presence of melanosomes in varying stages of melanization in both cell types suggests that they have a common origin. This is a tumor of neural crest origin showing schwannian and perineurial differentiation, with ectopic production of melanin granules.—Key words: bovine, melanotic neurofibroma, S100 protein.

In human beings, melanotic schwannoma is a relatively rare, usually benign, neuroectodermal tumor [9], and is considered to be a variant of schwannoma [2]. Compared with this tumor, there were only few reports concerned with melanotic neurofibroma [18]. Melanin synthesis was demonstrated in a human neurofibroma, but it was unclear whether the tumor cells were pigment-synthesizing Schwann cells or whether they originated from a coexisting melanocytic tumor [10]. In dogs, two cases of malignant melanotic schwannoma have been studied histologically and ultrastructurally [16]. This report describes a case of bovine melanotic neurofibroma.

A raised tumor mass, 10 × 10 × 5 cm, was detected near the right ear of an 8-month-old Holstein steer. At the age of 17 months, the tumor became enlarged to 35 × 25 × 15 cm, extending from the head to the right cheek (Fig. 1). A part of the tumor was excised for histological examination, and was dark brown in color and gelatinous. Because the skin surface became partially ulcerated and infected, the animal was brought to an abattoir 2 months later. Macroscopically, the subcutaneous tumor was unencapsulated, and soft or gelatinous but partly firm. Its cut surface showed black, brown or white tissues, and they were intermingled in varying proportions.

Tissue samples from the excised and necropsied tissues were fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin (HE), Fontana-Masson, trichrome, and silver stains. The avidin-biotin-peroxidase complex (ABC) method was applied to paraffin wax sections. Polyclonal antibodies to S100 protein (Nichirei, Tokyo, Japan) and glial fibrillary acidic protein (GFAP) (Lipshaw, Detroit, U.S.A.) and monoclonal antibodies to neurofilament protein (NF) (Dako A/S, Glostrup, Denmark) and vimentin (Dako, Carpinteria, U.S.A.) were used as primary antibodies, and a universal kit (BioGenex Laboratories, San Ramon, U.S.A.) was used in the subsequent processes. Small pieces from formalin-fixed tissues were postfixed in osmium tetroxide, and embedded in epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate, and viewed with an electron microscope.

Histologically, the partially resected tumor was situated in the subcutaneous tissue. It extended into the dermis, and was ill-defined from the surrounding normal tissue. The neoplastic cells were arranged disorderly or in short fasciculi, and the intercellular stroma was rich in reticulin fibers with or without mucinous material. The most predominant cells had round to oval nuclei and round to long oval cell bodies with cytoplasmic processes, and spindle-shaped cells with fusiform nuclei were admixed with them. The nuclei were finely dispersed, and the nuclei had inconspicuous. There were intracytoplasmic pigment granules, stained positively with Fontana’s stain, in many neoplastic cells (Fig. 2). Mitoses were exceedingly rare. In necropsy specimens, the neoplastic tissue was similar to that in the resected tumor in morphology. In some areas, however, pigment laden cells were few in number or there was abundant collagen in the stroma.

Immunohistochemically, the tumor cells showed positive staining for vimentin, and numerous vimentin-positive cell processes were seen intercellularly. The vast majority of the predominant cells were variably positive for S100 protein (Fig. 3), whereas most spindle cells were negative. Granules were confirmed not only in S100-positive cells but also in S100-negative elongated cells. Of the predominant cells, many showed weak reactivity for GFAP but a few stained intensely, and almost all spindle cells did not stain. Except in normal nerve fascicles remaining in the tumor tissue, NF-positive neurites were not seen.

Ultrastructurally, the most predominant cells, characterized by branching cell processes, continuous external laminae, a few pinocytotic vesicles, and moderately to slightly developed organelles, resembled Schwann cells (Fig. 4), but numerous pinocytotic vesicles, discontinuous
external laminae or abundant intermediate filaments were detectable in some cells. Frequent cells contained variable numbers of solitary stage IV melanosomes, occasionally with stage II, III or compound melanosomes (Fig. 5). The round to ovoid nuclei were almost devoid of heterochromatin, and desmosome-like structures were extremely rare.

The other types of cells resembled perineurial cells or fibroblasts. These cells had spindled nuclei with slightly condensed chromatin. There were poorly developed organelles and numerous pinocytotic vesicles in the cytoplasm, and small numbers of melanosomes were at times seen (Fig. 6). Cell processes were slender and unbranched, and fragmentary external laminae could be discerned in neoplastic perineurial cells, but not in fibroblastoid cells.

Human neurofibromas result from proliferation of several cell types including Schwann cells, perineurial cells and fibroblasts, and the latter two were S100 negative [6]. Although schwannoma and neurofibroma had been placed in the same category in domestic animals [3], immunohistochemistry demonstrated that they were distinct in cattle [15]. In the tumor described here, encapsulation was absent, and considerable numbers of S100-negative neoplastic cells existed. Such findings, which have been
observed in a bovine neurofibroma but not in a schwannoma [15], led to the diagnosis of a neurofibroma. Ultrastructural demonstration of both schwannian and perineurial differentiation also supported the diagnosis. In human beings, benign nerve sheath tumors could be classified by electron microscopy into schwannoma, neurofibroma and perineurinoma [5].

In human neurofibromas, electron microscopy disclosed the presence of three types of cells, namely, Schwann cells, perineurial cells, and cells intermediate between fibroblasts and perineurial cells [7], and the intermediate cells seemed to be modified neoplastic perineurial cells. In the present tumor, fibroblastoid cells containing pinocytotic vesicles but lacking external laminae were considered to correspond to the intermediate cells [7, 12]. The presence of solitary melanosomes at various stages of development in the cytoplasm of neoplastic perineurial cells is indicative of melanin synthesis by these cells [2, 4], and this fact supports the view that neoplastic Schwann cells and perineurial cells have a common origin [7, 12]. Since melanocytes originate from neural crest cells, it is probable that other types of cells of crest origin show ectopic production of melanin [16]. In humans, melanogenesis has been observed in tumors of neuroepithelial tissue such as astrocytoma [11] and ependymoma [13] as well as in nerve sheath tumors and meningioma [17], and it is certain that some tumors composed of cells of neural crest or neural tube origin are capable of producing melanosomes or melanin granules [4].

Fig. 4. A neoplastic Schwann cell has a forked process, in which stage IV melanosomes are present (arrow). A continuous external lamina invest this cell and cell processes (arrowheads). × 7,500.

Fig. 5. In a neoplastic Schwann cell, there are discrete melanosomes, two of which are stage II (short arrows). The external lamina is fragmented (long arrow), and a few pinocytotic vesicles are recognizable (arrowheads). × 12,000.

Fig. 6. A stage III melanosome (arrow) and numerous pinocytotic vesicles (arrowheads) are present in a fibroblastoid cell. × 15,000.
Although neither a schwannoma nor a neurofibroma expressed GFAP in cattle [15], neoplastic cells were weakly positive for GFAP in a malignant peripheral nerve sheath tumor of a cow [19]. Likewise, neoplastic Schwann cells revealed GFAP positivity in our case. In humans, occasional Schwann cells express GFAP, as do some schwannomas and neurofibromas [6]. In addition, GFAP-positive tumor cells were found in human melanotic schwannomas [14]. Because GFAP immunoreactivity has been reported in Schwann cells of autonomic nerves of the bovine iris [1], the presence of GFAP in a bovine melanotic neurofibroma is hardly surprising. Such a tumor is readily distinguishable from neoplasms arising from melanocytes [8].

REFERENCES