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NOTE

Angioleiomyoma in a conger (*Conger conger*)

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ABSTRACT: A wild adult male conger *Conger conger*, captured by a SCUBA diver in the waters of coastal Italy, was sent for laboratory analysis due to the presence of multiple productive ulcerous skin lesions localized in the dorso-lateral body area, caudally to the gill operculum under the dorsal fin. The main mass was sessile, ulcerated and hemorrhaging in appearance and was surrounded by several smaller masses, which originated peripherally from the same mass or were isolated, always with a tendency to ulceration. Histology confirmed that the newly formed tissue originated from derma invading the closer tissues. The tumour consisted of spindle cells, each with an ovoidal nucleus and rarely with evident nucleoli, which were arranged in parallel or storiform patterns and were often surrounding blood-filled spaces discontinuously lined by endothelial cells. Tumour tissue was richly vascularized and no mitoses were seen. The overlying epidermis was ulcerated. Masson's trichrome technique indicated the presence of a small amount of perivascular connective tissue. No excessive glycogen storage, bacteria, virus or fungi were detected by periodic acid-Schiff (PAS)-reaction. Immunohistochemistry showed dot-like or diffuse cytoplasmic positivity against smooth muscle actin and the monoclonal antibody D2-40. CD34 exhibited relevant immunoreactivity at plasma membranes. Growth fraction evaluated using MIB-1 was <1%. Immunoreactions for wide spectrum CK, CK5/6, CK8, CK18, EMA, desmin, myoglobin, S-100, CD20, CD68, GFAP, and NSE were negative. Histopathological and immunohistochemical results supported a diagnosis of angioleiomyoma, a benign tumour of the muscular cellular component of the blood vessels. To our knowledge, this is the first report of such neoplasms in fishes in which monoclonal antibodies work on fish tissues, facilitating a useful immunohistochemical approach for differential diagnosis.

KEY WORDS: Angioleiomyoma · Conger · Immunohistochemistry

INTRODUCTION

Angioleiomyoma, or vascular leiomyoma, is a rare, benign tumour of the smooth muscle cells of blood vessels that has occasionally been described in animals as well as in humans (Liu & Mikaelian 2003, Gonzales Lavandeira et al. 2016). This tumour is generally characterized by spindle cells differently arranged in parallel or interwoven bundles, and contains veins in the inner core; arteries have only rarely been reported inside. For veterinary pathologists, the main problem with this kind of neoplasm is to precisely identify the cell origin, as it is similar to some other vascular and nervous tumours. This is particularly true for the spindle cell tumours, such as schwannoma, neurofibroma, leiomyoma, fibroma and occasionally rhabdomyoma. Immunohistochemistry and ultrastructure have been suggested as possible tools to identify this tumour type and to distinguish it from similar tissue changes (Une et al. 2010).

The European conger *Conger conger* (Pisces: Congridae) is the largest eel in the world. This species is native to the northeastern Atlantic, including the Mediterranean Sea (Busalacchi et al. 2010).

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Few sporadic data are available in the literature concerning diseases of the conger, most of which are in reference to parasites (Costa et al. 2009). Only a single kind of tumour, a fibrosarcoma, has been described in this species (Mawdesley-Thomas 1972). The aim of the present study was to describe the first record of angioleiomyoma in fish, and to address the immunohistochemical features useful for characterization of this tumour.

MATERIALS AND METHODS

A wild adult male conger (130 cm, 1.3 kg) was captured by a SCUBA diver in the coastal waters close to Augusta (Siracusa, Italy). The fish was sent to the Centre for Experimental Fish Pathology of Sicily (CISS) because of the presence of protruding and ulcerated skin. The specimen was photographed, measured, and a necropsy was performed. Tissue samples were collected for histological examination and fixed in a 10% seawater formalin solution for 12 h at room temperature (RT). After re-washing in tap water, samples were rinsed in graded alcohol solutions and later cleared in xylene. Following paraffinization, tissue samples were embedded in paraffin wax at 56°C, then 5 µm serial histological sections were obtained from a microtome. Sections were stained using haematoxylin-eosin, periodic acid-Schiff (PAS), Sirius Red and Masson's trichrome techniques. Immunohistochemical analysis was carried out on parallel xylane-coated slides. In detail, sections were treated in a moist chamber (1) with 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity (30 min at RT); (2) with normal sheep serum to prevent unspecific adherence of serum proteins; (3) with the following monoclonal primary antibodies against D2-40 (clone D2-40, working dilution [wd] 1:50), CD34 (clone QBEnd10, wd 1:50), wide spectrum CK (clone AE1/AE3, wd 1:50), CK5/6 (clone D5/16B4, wd 1:50), CK8 (clone 35bH11, wd 1:50), CK18 (clone DC10, wd 1:50), EMA (clone E29, wd 1:300), smooth muscle actin (SMA; clone 1A4, wd 1:200), desmin (clone D33, wd 1:50), myoglobin (clone MYO18, wd 1:50), CD20 (clone L26, wd 1:500), CD68 (clone KP1, wd 1:500), GFAP (clone 6F2, wd 1:200), NSE (clone BBS/NC/VI-H14, wd 1:800), and Ki-67 (clone MIB-1, wd 1:200) (all commercially obtained from DakoCytomation) or with polyclonal antibody against S-100 (DakoCytomation, wd 1:300); (4) with sheep anti-mouse or anti-rabbit immunoglobulin antiserum (Behring Institute; wd 1:25; 30 min at RT); and (5) with mouse/rabbit anti-horseradish peroxidase-antiperoxidase complexes (DakoCytomation; wd 1:25; 30 min at RT). For the demonstration of peroxidase activity, the sections were incubated in darkness for 10 min with 3-3′ diaminobenzidine tetra-hydrochloride (Sigma Chemical), 100 mg of diaminobenzidine in 200 ml 0.03% hydrogen peroxide in phosphate-buffered saline (PBS). The nuclear counterstaining was performed using Mayer's haemalaun. In addition, human tissues such as cutaneous, mesothelial, glial, lymphatic, striated and cardiac muscular fragments were utilized as tissue-positive controls for the abovementioned antisera. To test the specificity of each immunostaining in order to rule out the possibility of a non-specific reaction, serial sections of each specimen were tested by replacing the specific antisera by either PBS or normal rabbit serum, thus obtaining negative controls.

RESULTS

The multiple protruding ulcerous skin lesions were localized in the back lateral body areas of the fish, 15 cm caudally to the gill operculum and 10 to 12 cm lateral to the dorsal fin. The main mass was an ulcerated, haemorrhaging bulge and was surrounded by several smaller masses that either originated peripherally from the main nodule or were isolated, always with tendency to ulceration Fig. 1. At low magnification, the epidermis showed discontinuity and appeared ulcerated (Fig. 1b). Histology confirmed that the newly formed tissue originated from the dermis invading surrounding tissues. The tumour was characterized by spindle cells, each with an ovoid nucleus and rarely with evident nucleoli, which were arranged in parallel or storiform patterns often surrounding vascular structures (Fig. 2a). Tumour tissue was richly vascularized (Fig. 2b) and no mitoses were seen. Masson's trichrome technique revealed a small amount of perivascular connective tissue, the collagen nature of which was confirmed by Sirius Red staining. Neither glycogen storage nor mucus apical production or microbial and fungal amicroorganisms in the tumour were detected by PAS reaction. Immunohistochemistry showed dot-like or diffuse cytoplasmic positivity with SMA (Fig. 3a) and D2-40 (Fig. 3b). As a usual surface marker, CD34 exhibited intense immunoreactivity with plasma membrane (Fig. 3c). Growth fraction evaluated using MIB-1 was <1%. Immunoreactions for wide spectrum CK, CK5/6, CK8, CK18, EMA, desmin, myoglobin, S-100, CD20, CD68, GFAP, NSE were always negative.

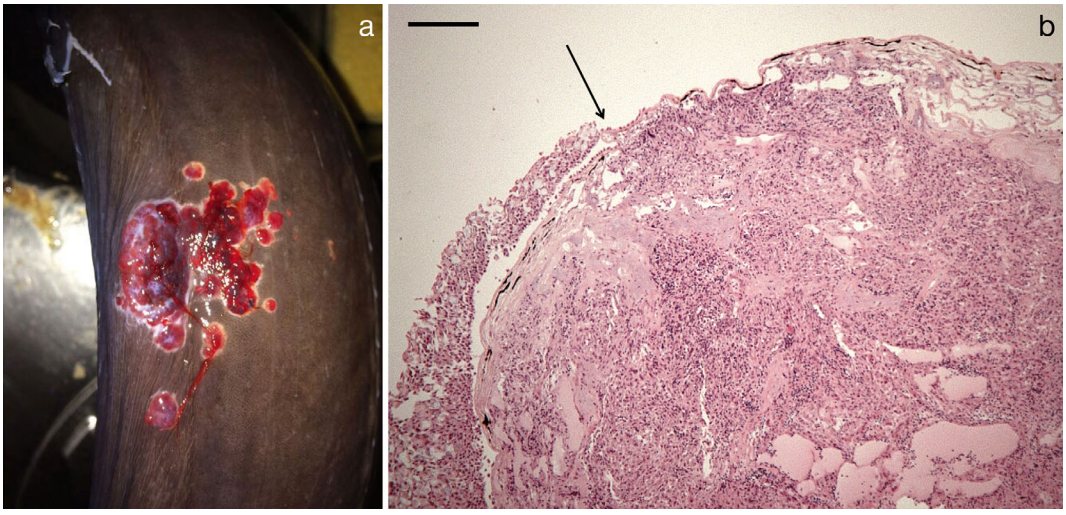

Fig. 1. *Conger conger*. (a) Macroscopical aspect of the neoplastic tissue found in a conger showing the main larger mass surrounded by several smaller nodules with the overlying skin ulcerated, and (b) low magnification of the epidermis, showing discontinuity and ulceration (arrow). H&E. Scale bar = 100 µm

DISCUSSION

The histopathological and immunohistochemical results of the present study supported a diagnosis of an angioleiomyoma (vascular leiomyoma), a benign tumour of the muscular cellular component of the blood vessels (Weiss & Goldblum 2008). As far as we know, this is the first report of such neoplasia in fish and only the second tumour described in conger (Mawdesley-Thomas 1972), whilst in European eels *Anguilla anguilla*, an additional 2 tumours have recently been described (Marino et al. 2010a, Gjurčević et al. 2014).

Angioleiomyomas originate from smooth muscle cells of the blood vessel walls (Hendrick et al. 1998). Stout (1937) published the first report of this tumour in humans, where it is generally reported in lower limbs, knees and rarely the head and trunk (Hachisuga et al. 1984). In animals, angioleiomyomas have been reported in dogs (Carpenter & Hamilton 1995, Katsuta et al. 1998), cats (Liu & Mikaelian 2003), monkeys (Gozalo et al. 2010), degus (Jakab et al. 2010) and cattle (Une et al. 2010). Angioleiomyomas commonly arise in deep dermal or subcutaneous tissues (Holst et al. 2002). Histologically, these lesions are characterized by a various amount of smooth muscle fibers surrounding vascular channels and are peripherally lined by a thin fibrous capsule. Morimoto (1973) described 3 histological variants in humans: solid, cavernous and venous, each with specific clinical–pathological characteristics that are also sex-linked. The solid or capillary form is the most common and generally originates in lower limbs in females (Holst et al. 2002). The histo-

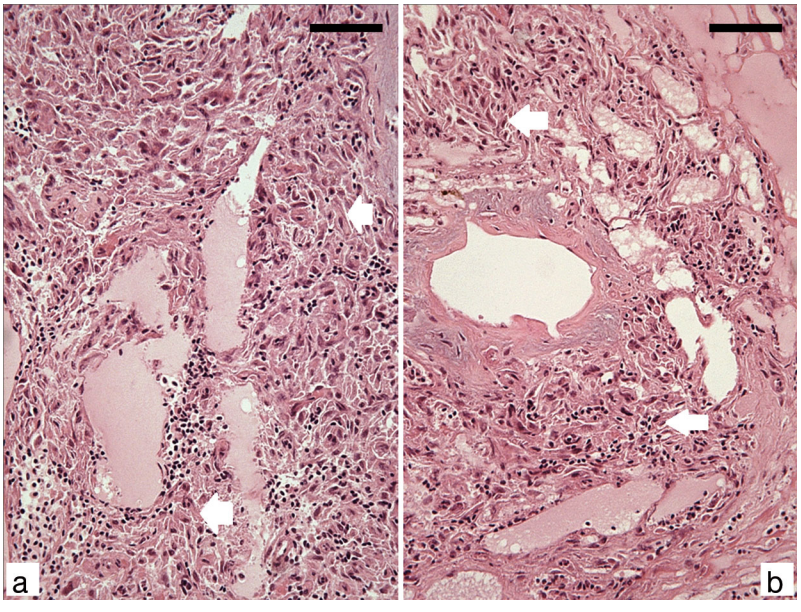

Fig. 2. *Conger conger*. Neoplastic proliferation in a conger composed of (a) spindle cells (white arrows) containing an ovoid nucleus, arranged in parallel or storiform patterns and (b) abundantly vascularized peritumoral tissue. H&E. Scale bar = (a,b) 50 µm

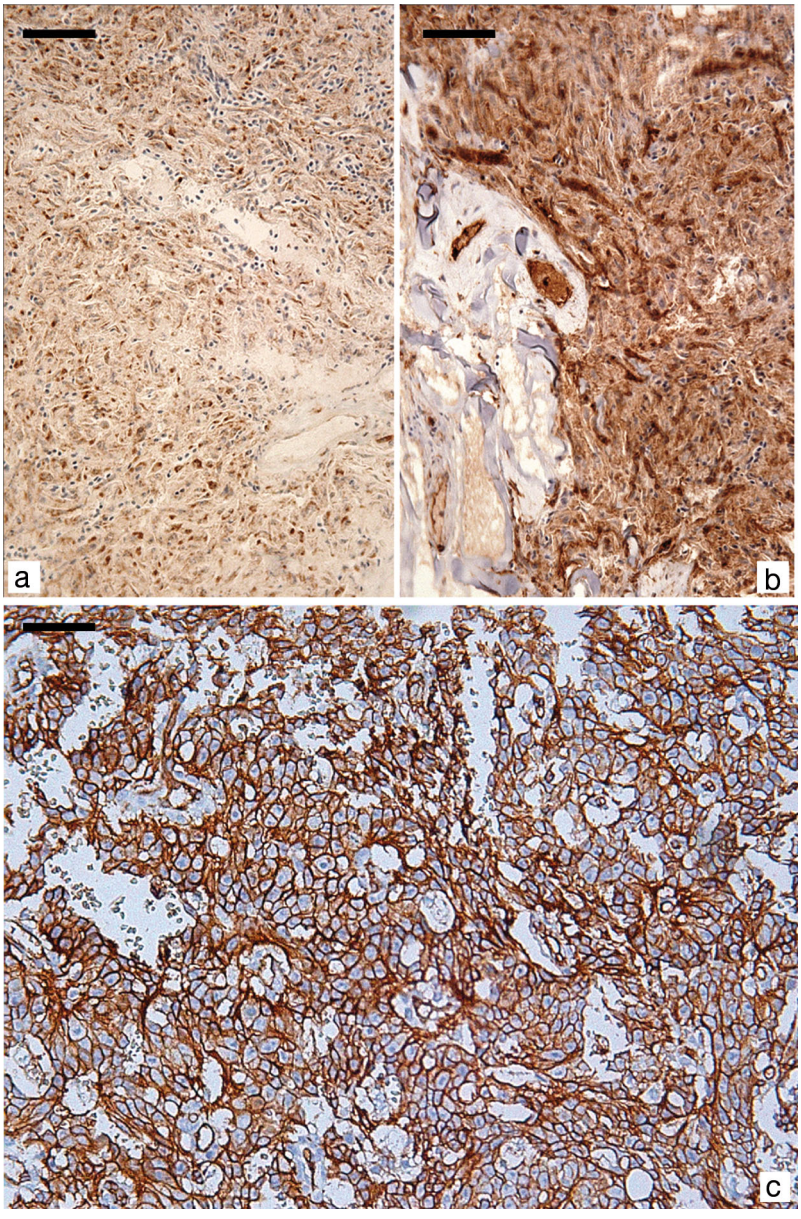

Fig. 3. *Conger conger*. Immunohistochemical analysis of tumour, showing (a) cytoplasmic positivity with smooth muscle actin (SMA) and (b) a diffuse pattern with D2-40; (c) CD34 showed an intense immunoreactivity with plasma membranes. Mayer's haemalaun counterstain. Scale bar = (a,b) 50 µm, (c) 30 µm

logical pattern of the current case in the conger indicates that the lesion was the solid form. The immunohistochemical approach allows characterization of neoplastic vascular growth, permitting differential diagnoses from other vascular tumours, such as glomus tumours, haemangioma or angiolipoma (Porter et al. 1991, Sciot et al. 1997). However, other neoplastic entities such as peripheral nervous system tumours (PNSTs), ganglioneuromas and traumatic neuromas should also be differentiated from angioleiomyomas, and this could be difficult with diagnoses based exclusively on histology. In particular, the spindle cells of angioleiomyomas have no expression of the S-100 protein with concurrent expression of SMA — reactions that are not expected in schwannoma (nerve sheath tumors that develop from Schwann cells; Marino et al. 2007, 2008, 2012). Similar differences could be considered even to differentiate neurofibroma (Marino et al. 2010b). No mature adipose cells were present in the examined angioleiomyoma, in contrast to what has been reported from angiomyolipoma (Galofaro et al. 2005).

In conclusion, our results confirm that certain neoplastic disorders in animal species, particularly in fish, require specific immunohistochemical tools for a more robust classification. Moreover, the point that commercially available monoclonal antibodies, originally produced for the study of human tissue, can be successfully applied to other species using piscine material must be highlighted, although their possible negative expression must be verified each time with positive controls, even when using human tissue or tumours.

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