Short communication

Cutaneous Leiomyoma in a Goldfish *Carassius auratus*

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(Received February 3, 2015)

ABSTRACT—This study describes the histopathological features of a cutaneous leiomyoma in a goldfish. A mass, measuring $3.5 \times 2 \times 1$ cm, located dorsolaterally along the cranial lateral line above the right operculum. The mass was sessile and golden orange with hemorrhagic foci. Histological examination revealed interlacing bundles of spindled to elongated tumor cells with cigar-shaped nuclei with no malignancy. The tumor cells showed red color with Masson's trichrome stain. Immunohistochemically, the tumor cells were positive for vimentin, desmin and α -smooth muscle actin, but not for S100. Based on these findings, this case was diagnosed as a cutaneous leiomyoma.

Key words: cutaneous leiomyoma, *Carassius auratus*, goldfish, immunohistochemistry

Leiomyomas, tumors of smooth muscle cells, can arise from any organ with a mesenchymal component or smooth muscle, primarily the urogenital system followed by the gastrointestinal system and the skin in human beings and animals (Pfeiffer and Asashima, 1990; Holst *et al.*, 2002; Liu and Mikaelian, 2003; Tomas *et al.*, 2009). Leiomyoma is uncommon in fishes (Sarkar *et al.*, 1955; Ciltas and Hisar, 2005). In one review of the literature regarding fish tumors by Mawdesley-Thomas in 1975, there were seven reports of leiomyoma mostly from the gastrointestinal tract. Furthermore, Roberts (1989) reported an eight percent prevalence of the testicular leiomyoma in yellow perch from the Great Lakes in Canada. Herein, we described the gross, histological and immunohistochemical features of leiomyoma in a goldfish.

Materials and Methods

A five-year-old female goldfish, *Carassius auratus*, with a length of 21 cm, was presented with a solitary mass protruded from just behind the operculum or gill cover near the lateral line on the right side of body (Fig. 1). The mass developed forward, covered the operculum and passed it toward the head, but the grown-edge of the mass was rounded and free and did not have any adherence to the head. The golden-orange colored mass had dimension of about $3.5 \times 2 \times 1$ cm which showed no discoloration of the skin. It was sessile, soft and non-ulcerative with regular and smooth edges and devoid of scales. It appeared well circumscribed, but not encapsulated. Several foci of hemorrhages were observed on the surface of the mass. Necropsy showed no abnormality, tumor or visceral metastasis in other sites. The tissue samples obtained were fixed in 10% neutral buffered formalin, dehydrated with graded ethanol, cleared by xylol and embedded in paraffin wax. The sections of $5 \mu\text{m}$ in thickness attained by microtomy were stained with hematoxylin and eosin (HE) and also with Masson's trichrome to differentiate between collagen and muscle fibers. In addition, immunohistochemical staining of additional serial sections was performed with avidin-biotin complex (ABC) method using antibodies to vimentin (1:100, DAKO), desmin (1:100, DAKO), α -smooth muscle actin (α -SMA) (1:10, BioGenix), and S100 (1:400, DAKO). The sections related to immunohistochemical evaluation were counterstained with Harris hematoxylin. Moreover, antigen retrieval was performed for 13 min in citrate buffer solution (pH 6.0) following deparaffinization and rehydration. In addition, slides were blocked for endogenous peroxidase with 3% H_2O_2 for 20 min.

Results

Histologically, the tumor was composed of the spin-

Fig. 1. A solitary mass, measuring $3.5 \times 2 \times 1$ cm, attached along the right cranial lateral line, dorsolateral to the right operculum, is depicted. The mass is elongated in morphology measuring 3.5×2 cm.

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Fig. 2. (A) Fascicles of spindle to elongated cells with cigar-shaped nuclei mimicking normal smooth muscle cells are seen in this microphotograph. (B) The elongated mesenchymal cells which tended to be arranged in fasciculi or interlacing bundles with the hemorrhage (arrow heads) and hyperemia (arrows) foci are present in the dermis. (C) Histochemically, Masson's trichrome stain differentiating red smooth muscle fibers from blue collagen ones. Immunohistochemically, the tumor cells were positive for vimentin (D), desmin (E) and α -smooth muscle actin (F), scale bar 50 μ m (B) and 12.5 μ m (A, C, D, E, and F).

dled to elongated cells with a parallel orientation in interlacing bundles or occasionally with a whirling pattern (Fig. 2A and B). The tumor cells had small amount of eosinophilic cytoplasm with indistinct cell borders and lacked cross striations. The nuclei containing inconspicuous nucleoli were elongated, cigar-shaped with blunt or rounded ends (Fig. 2A). Mitotic figures, pleomorphism or necrosis were absent. In addition, no invasion and metastases of the tumor cells were observed. The surface epithelium was intact and the underlying neoplastic cells had no encroachment into the epithelium and vice versa. There were remarkable hemorrhages (arrow heads) and hyperemia (arrows) in the neoplastic tissue (Fig. 2B). Masson's trichrome staining that tumor cells and normal muscle fibers surrounding tumor tissue showed in red color, and small amounts of collagen fibers were observed in stroma (Fig. 2C). The immunohistochemically, tumor cells were positive for vimentin (Fig. 2D), desmin (Fig. 2E) and α -SMA (Fig. 2F), but not for S100. The histopathological findings were consistent with the diagnosis of cutaneous leiomyoma.

Discussion

Leiomyoma has a histological appearance similar to other spindle cell tumors such as fibroma, fibrous histiocytoma, neurofibroma, schwannoma, rhabdomyoma, hemangiopericytoma and leiomyosarcoma (Morales and Schmidt, 1991; Cooper and Valentine, 2002; Namazi *et al.*, 2014). Therefore, they must be taken into consideration for differential diagnosis. Distin-

guishing between these tumors may be difficult by routine histological methods, but immunohistochemical analysis with antibodies against intermediate filaments may be helpful for accurate diagnosis of these tumors. In the current case, a mesenchymal origin of the tumor was confirmed by positivity for vimentin. Desmin detects cytoskeletal proteins specific for muscle differentiation, but it does not distinguish among tumors of smooth, cardiac, and skeletal muscles (Cooper and Valentine, 2002; Liu and Mikaelian, 2003). Desmin shows diffuse positivity in leiomyomas, whereas the fibromatous tumors are typically negative or only focally positive (Tomas *et al.*, 2009). Since the tumor cells were diffusely positive for desmin, we could exclude non-myogenic tumors from the list of differential diagnosis. In addition, α -SMA is a specific marker for smooth muscle differentiation which can distinguish smooth muscle tumors from tumors of the skeletal or cardiac muscle (Liu and Mikaelian, 2003). S100 is marker for diagnosis of melanocytic tumors, neurofibroma, peripheral nerve sheath tumors, and schwannoma (Cooper and Valentine, 2002; Holst *et al.*, 2002). The tumor cells of the present case showed no S100 expression which made us to differentiate it from tumors originating from neural crest. Hemangiopericytoma was ruled out as the tumor classification because it lacked the perivascular whorls of fusiform cells which are the hallmark of this neoplasm (Morales and Schmidt, 1991; Namazi *et al.*, 2014). In addition, the tumor cells in the present case did not show atypia, mitotic figures, invasion and metastasis as well as tumor necrosis, which are the most important criteria in distinguishing leiomyo-

mas from leiomyosarcomas (Cooper and Valentine, 2002; Patton *et al.*, 2006). Although leiomyoma should lack metastatic potential, metastases of the tumor to the lungs followed by lymph nodes have been reported (Patton *et al.*, 2006). Leiomyomas have been reported from stomach, mouth, skin, head, fins and testicles of fishes (Schlumberger, 1949; Budd *et al.*, 1975; Herman and Landolt, 1975; Pfeiffer and Asashima, 1990; Ciltas and Hisar, 2005). A review of the veterinary literature indicated two cases of leiomyoma on the head of goldfishes, one reported by Ciltas and Hisar (2005) and another one by Plehn as cited by Mawdesley-Thomas (1975). In addition, in a study conducted by Schlumberger (1949), around 3 to 4 percent of the goldfishes in a large pond suffered from single or multiple cutaneous leiomyoma. The tumors were located in scattered regions of the trunk, top of the head, and all fins. Among those arising on the trunk, three large tumors were located immediately behind the operculum as noted in this case. However, according to Schlumberger (1949) contrary to this case, the surface epithelium upon the neoplastic tissue sent long pegs into the tumor. In addition to the goldfishes, leiomyomas have been reported from other fish species (Sarkar *et al.*, 1955; Herman and Landolt, 1975; Roberts, 1989). Kubota (1955) found leiomyoma on the lower jaw of oral cavity in a rainbow trout (*Salmo irideus* Gibbons) composing with twelve polyps developing from subserosa between the oral epithelium and ceratohyal bone. In another study, Sarkar and colleagues (1955) reported a leiomyoma on the fins of an Indian catfish. Herman and Landolt (1975) reported a case of leiomyoma in testis of a largemouth bass developing from smooth muscle in the interstitial tissue of the testis. According to the medical literature, the muscle bundles of the blood vessels particularly the veins have been accepted as origin of the majority of solitary cutaneous leiomyomas, whereas multiple ones derive mostly from the arrectores pilorum muscles of the skin (Stout, 1937; Holst *et al.*, 2002). It is more likely that these tumors develop in the walls of the veins rather than arteries (Stout, 1937). Although the source of the neoplastic smooth muscle cells of the present case remains unknown, this tumor may originate from the smooth muscle cells of the blood vessels. In conclusion, given the widespread use of goldfishes in the aquariums for ornamental purposes, they should be accurately considered in terms of the presence of such tumors.

Acknowledgments

The authors would like to thank Mrs. S. Jowkar from the Department of Pathology of the Veterinary School, Shiraz University and Mrs. F. Haddad from the Daneshbod Pathology Laboratory, Shiraz, Iran, for their technical assistance.

Competing interests

The authors declare that they have no competing interests in this research work.

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