Borderline ovarian mucinous cystadenoma with invasion of stroma in the goldfish *Carassius auratus* (L.)

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Abstract

We report an ovarian cystadenoma in an adult goldfish. The standard histopathologic examination of the tumour mass contained areas of mature cystic tissue that exhibited typical tall columnar cells with basally situated nuclei and abundant intracellular mucins stained with Alcian blue. Sections of the tissue showed an invasive stromal implant of borderline mucinous cystadenoma associated with clusters of adenocarcinoma-like cells. The immunohistochemical studies showed the typical immunohistochemical pattern of staining of CK7 found in classical mucinous cystadenoma. The invasive stromal implant also showed positive staining with the CK7 antibody. The utility of cytokeratin 7 (CK7) expression is restricted and allows ovarian mucinous adenomas to be differentiated from lower gastrointestinal neoplasms, as the extent of staining for CK7 has been shown to be diagnostically relevant for the distinction of primary and secondary mucinous tumors of the ovary.

Introduction

Teleost fishes constitute the largest and most diverse class of vertebrates, with over 20,000 known species. Their diversity and placement in the phylogenetic tree make them ideal subjects for comparative carcinogenesis studies, providing broader knowledge about basic mechanisms, in comparison to studies limited only to mammalian models. Previously, such studies have primarily dealt with tumors in the higher vertebrates (mammals and bird); considerably less attention has been devoted to the lower vertebrates (fishes, amphibians, and reptiles). Currently, the classification of fish neoplasms is largely based on mammalian criteria (Masahito et al., 1998; Romano and Marozzi, 2004; Romano et al., 2010).

All the major groups of tumours that occur in mammals and birds have been reported in fishes. Some very specific, well-documented neoplasias have been described in fish, including viral-induced dermal sarcomas in walleye,

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Stizostedion vitreum (Mitchill), and aflatoxin-associated hepatocellular adenomas and carcinomas in rainbow trout, *Oncorhynchus mykiss* (Walbaum), in addition to a wide variety of sporadic tumours in numerous other species (Lee et al., 1971; Roberts, 2012).

Materials and methods

Massive abdominal enlargements were observed in an adult goldfish, *Carassius auratus* (L.) from an ornamental fish breeder (Santa Fe, Argentina). The fish, about two years of age, had a weight of 460 g and a total length of 343 mm. At necropsy, a tumour of 19 cm in diameter was found in the abdominal cavity. Fragments of the tumour were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 to 5 μm, and stained with haematoxylin and eosin and alcian blue. In addition histological sections were also stained using immunohistochemical procedures, according to a modified avidin-biotin-peroxidase complex technique.[2] This involved tissue slides being deparaffinised, rinsed with xylol and then rehydrated with alcohols of different grades (absolute ethanol, 90, 80, 70, 50%). The endogenous peroxidase activity was blocked by incubating the slides for 20 min in 0.3% H_2O_2 in a 5% methanol solution. After washing the slides in water and PBS/0.05%-Tween 20 solution, they were incubated in normal 1/100 serum[a], in a 10% PBS bovine serum albumin (BSA) solution at normal temperature for 30 min in a humid chamber. After incubation, the primary anti- cytokeratin 7 (CK7) antibody[b] was added at a dilution of 1:200 in PBS-BSA 10%, and the slides were incubated overnight at 41°C in humid chamber. This was followed by rinsing and incubation for 7 min in a 50 mL 30.3-diaminobenzidine solution containing 1% PBS-BSA in 50 mL H_2O_2[c]. Counterstaining was then performed with haematoxylin (Hsu et al., 1981).

Results

The tumour was cystic and multilobulated with abundant mucins (Figure 1). Histopathological examination showed the tumour mass contained areas of mature cystic tissue that exhibited typical tall columnar cells with basally situated nuclei and abundant intracellular mucins stained with Alcian blue (Figure 2). Secondary cysts and short papillary infoldings were characteristic. In some cysts, a filigree of fine projections supported by delicate connective tissue was the predominant pattern.

A transitional zone was observed between the classical mucinous cystadenoma and papillary areas. Rather than a single layer of well-differentiated mucinous cells, the cysts were lined by atypical epithelium that was stratified into two to three layers. Sections of the tissue showed an invasive stromal implant of a borderline mucinous tumour associated with clusters of adenocarcinoma-like cells. The neoplastic epithelial structures were prominent and the tissue mass is not perforated (Figures 3 and 4). The immunohistochemical studies showed the typical immunohistochemical pattern of staining of CK7 found in classical mucinous cystadenoma. The invasive stromal implant also showed positive staining with the CK7 antibody (Figure 5).

Discussion

Neoplasms of goldfish and common carp, *Cyprinus carpio* L., are well represented in the literature. An important characteristic of this group of fish is their longevity, especially the koi strains of common carp. Goldfish are also well

Figure 1. Gross appearance of borderline mucinous cystadenoma of a multilocular cystic ovary with irregular walls with abundant mucins. Bar = 1 cm. Insert: *Carassius auratus* with massive abdominal enlargements. Bar = 4.5 cm
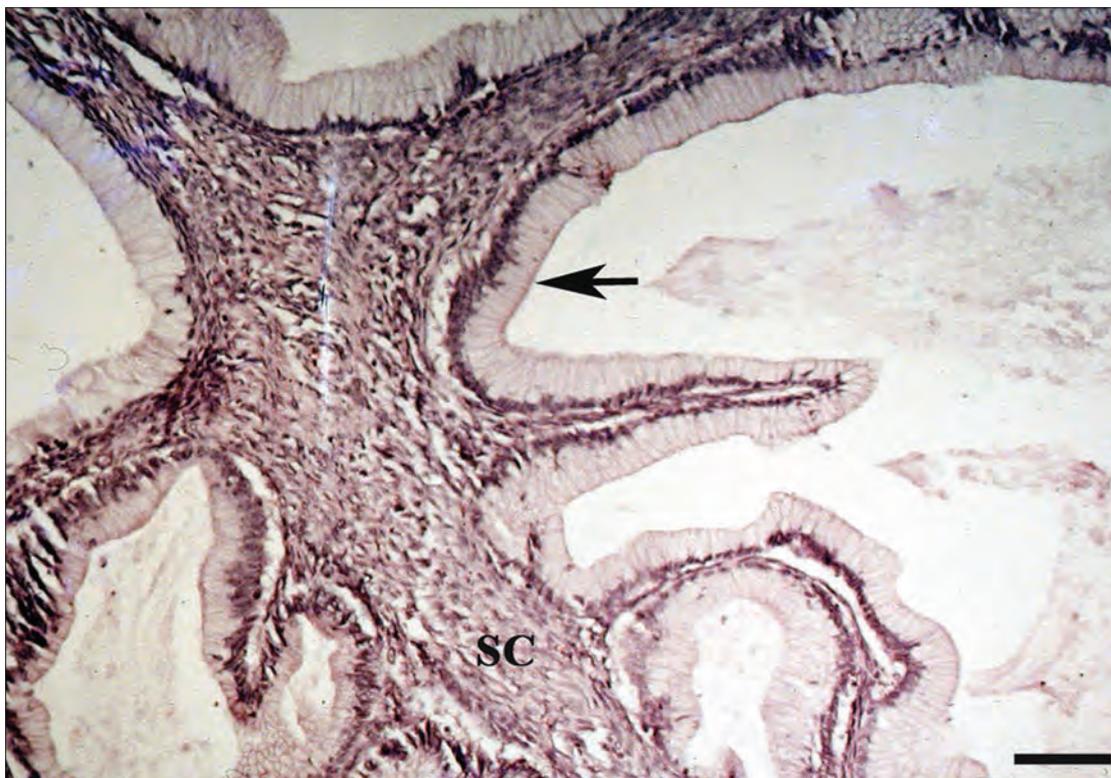

Figure 2. Mature cyst showing typical tall columnar cells with basally situated nuclei and abundant intracellular mucins (arrow) supported connective tissue (SC). Haematoxylin and eosin. Bar = 200 μm
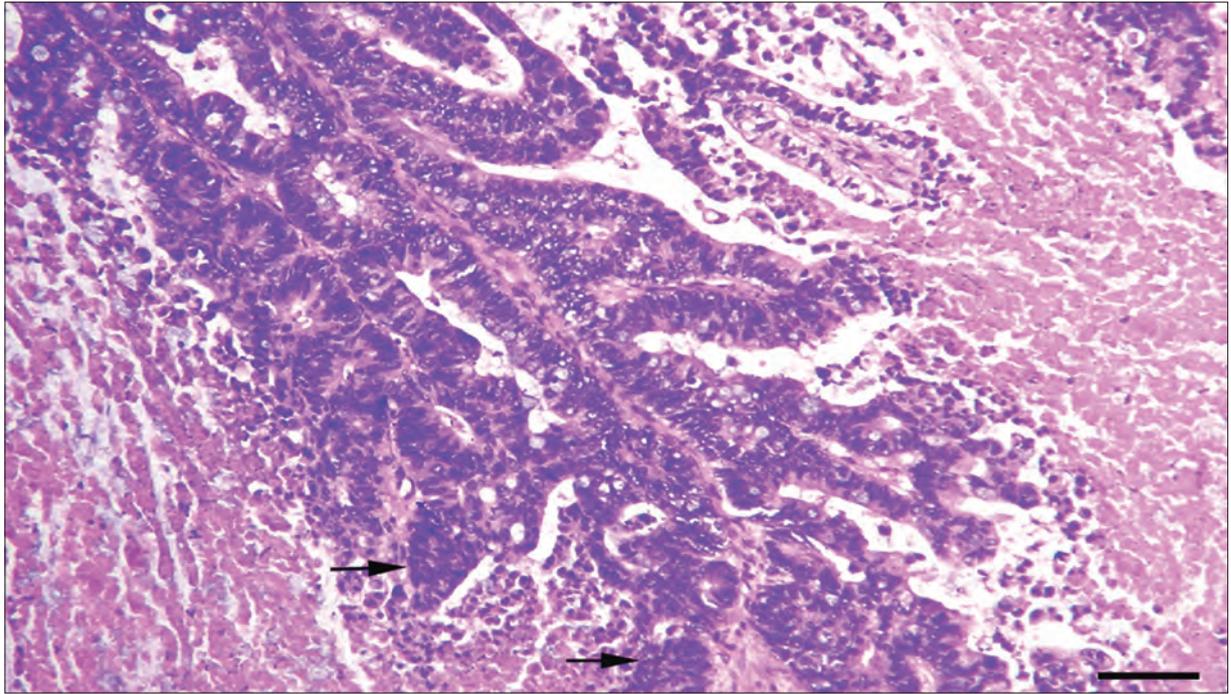
Figure 3. Microscopic appearance of borderline mucinous cystadenoma of the ovary. Epithelium is pleomorphic and forms small papillary processes with incipient invasion of stroma (arrows). Haematoxylin and eosin. Bar = 50 μm
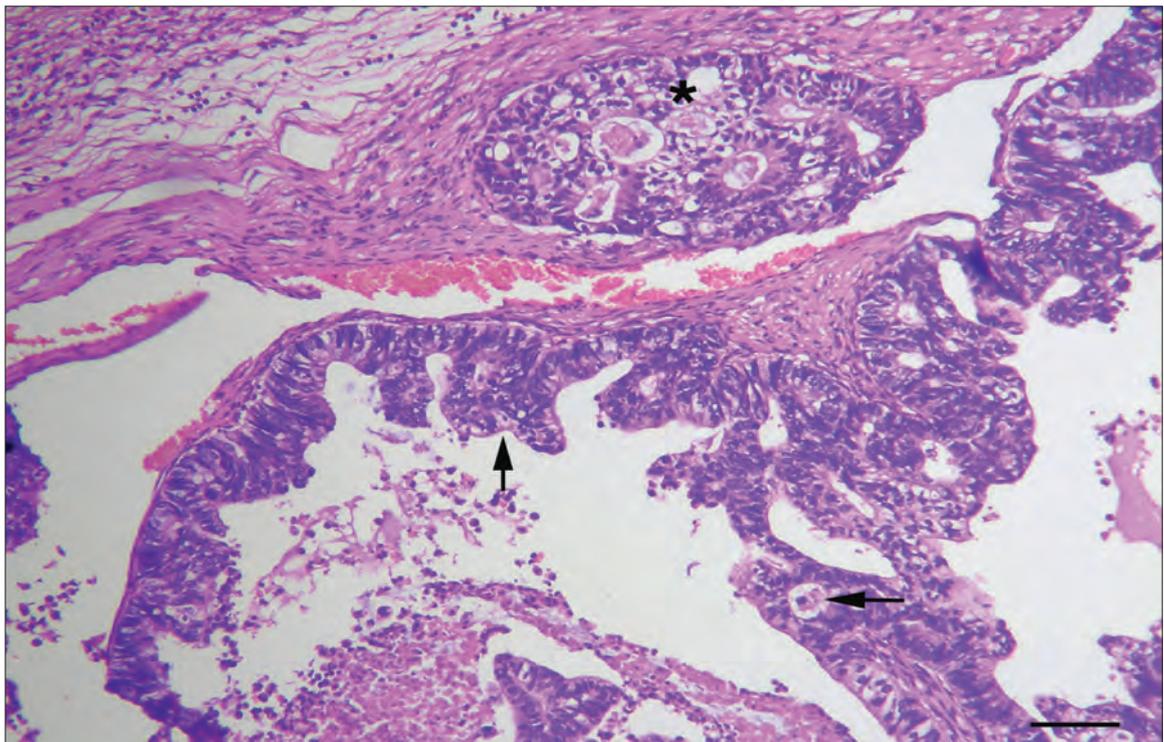

Figure 4. Borderline mucinous cystadenoma of the ovary showing atypical and hyperplastic epithelium (arrows) and invasion of stroma by clusters of adenocarcinoma cells (*) Haematoxylin and eosin. Bar = 100 μm
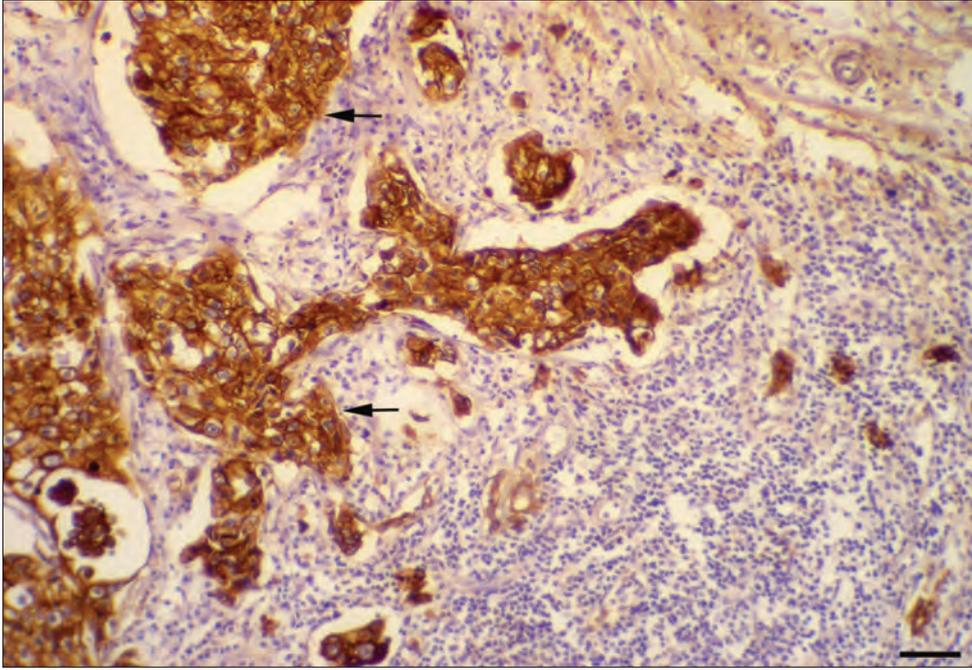
Figure 5. Stroma with clusters of adenocarcinoma cells exhibiting CK7 expression (arrows). Anti-CK7 with haematoxylin counterstain. Bar = 100 μm

adapted to environments that are suboptimal for other species of fish. A consequence of this adaptation is the ability to detoxify and repair damage caused by toxins that may be carcinogenic. The longevity of the species and this effect of toxic carcinogens may explain the significant number of tumors described in these animals.

Abdominal enlargement associated with ovarian neoplasia was previously described in eight 4-6-year-old adult carp living in breeding ponds of carp fisheries in northern Japan (Hart, 2005). These neoplasias were tentatively classified as dysontogenetic tumours of the ovary, possibly teratoid in nature. Another study described 21 ovarian neoplasias in ornamental hybrid carp (Ishikawa and Takayama, 1978). Histologically, these neoplasias exhibited considerable variation, but they were composed mainly of various types of cells similar to those of human dysgerminoma, granulosa or theca cell tumors

or embryonic carcinomas. Squamous cell nests were found with mesenchymal elements in one tumor, but no mucinous cystadenoma was observed. Thus, ovarian tumours in carp may have diverse histogenetic origins and most likely arise both from germ cells and from ovarian mesenchyme.

In human pathology, the differentiation of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumours (atypical proliferative/borderline and carcinoma) can be difficult because of their similarities in morphology.

Borderline cystadenomas of the ovary are of epithelial origin and represent a unique intermediate stage between benign cystadenomas and adenocarcinomas. They are separated from cystadenomas by the presence of cellular atypia and from high-grade malignant tumors by the

presence of destructive stromal invasion. Some borderline cystadenomas have a minor form of invasion designated as microinvasion (Acs, 2005).

The World Health Organization (*WHO*) classified these neoplasms in humans as "borderline tumours"; they have been too widely recognized as ovarian tumors of "low malignant potential" and as "proliferative ovarian tumours," terminology that was accepted by the WHO in the year 2000. Currently, the use of the designation "adenoma or cystadenoma of low malignant potential" is not recommended (Fritz, et al., 2000).

Recent studies have reported conflicting results in relation to the differentiation between primary ovarian mucinous tumours and metastatic mucinous carcinomas in the human ovary. The utility of cytokeratin 7 (CK7) expression is restricted and allows ovarian mucinous tumors to be differentiated from lower gastrointestinal neoplasms, as the extent of staining for CK7 has been shown to be diagnostically relevant for the determination of primary and secondary mucinous tumours of the ovary. Primary ovarian mucinous tumours were virtually always diffusely positive for CK 7 (98%) (McCluggage 2011). Ovarian neoplasms have been reported in fishes, but borderline mucinous cystadenoma of the ovary with invasion of stroma is uncommon, and this would appear to be the first reported case (Hart, 2005).

Acknowledgments

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