NOTE

Immunohistochemical patterns of a non-viral papilloma in Goldfish (Carassius auratus, L.)

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Abstract

An epithelial tumour of the skin is reported in a goldfish (Carassius auratus). Immunohistochemistry showed huge cytoplasmic positivity in tumour cells, confirming the histological diagnosis of papilloma. Although similar neoplasias have been described in fish and have been related to herpesvirus, virological examination was negative in this case.

Epithelial tumours, mainly benign forms, have frequently been reported in fish (Mawdesley and Thomas, 1972). Skin tumours are reported in cyprinids including common carp (Cyprinus carpio L.) (Schubert, 1964, 1966; Huang, 1993) and koi carp (Cyprinus carpio) (Sano et al., 1985). Papilloma has been described, among the others, in smooth dogfish (Mustelus canis) (Wolke and Murchelano, 1976), Atlantic salmon (Salmo salar) (Carlisle and Roberts, 1977; Carlisle, 1977), different flatfishes (Peters and Watermann, 1979), black bullheads (Lctalurus melas) (Grizzle et al., 1984), white suckers (Catostomu commersoni) (Hayes et al., 1990), brown bullheads (Lctalurus nebulosus) (Poulet et al., 1994), roach (Rutilus rutilus) (Korkea-aho et al., 2006a, 2006b) and European eel (Anguilla anguilla) (Marino et al., 2010). Etiology includes virus (Schubert, 1964; 1966; Winqvist et al., 1968; Carlisle, 1977; Sano et al., 1985; Huang, 1993; Herman et al., 1997; Graham et al., 2004), chemical agents (Black, 1983; Chen et al., 1996; Beckwith et al., 2000), skin mechanical injuries (Peters and Watermann, 1979) and some unknown factors (Wolke and Murchelano, 1976). Chlorinated waste waters have been considered a possible cause of orocutaneous papillomas tumour (Poulet et al., 1994).

A specific type of papilloma is frequently described in allogynogenetic crucian carp (ACC) (Lu et al., 2009), a hybrid fish Carassius auratus gibelio (female) x Cyprinus carpio var. singuonensis (male), obtained in China in 1980 and
considered amongst the most valuable farmed freshwater species. Such papilloma has generally been described, during the winter season, on scales, fins and gill opercula of two or more year old fish, showing an incidence of up to 90% of the fish reared in the same tank (Lu et al., 2009). Although mortality is always low, diseased fish lose their marketability causing severe economic loss for the farmers (Lu et al., 2009).

The present paper describes a tumour on the head of a goldfish including its immunohistochemical pattern and some consideration on possible etiology.

A five year old, adult male goldfish (Carassius auratus), resided in an ornamental tank in a hotel, close to Gaeta (LT, Italy). The fish was reared in the same tank with several other fish of the same species; the water of the tank was continuously renewed and was supplied by a water source. The fish developed a large nodular bulge, localized to the left frontal dorsal portion of the head. The neoplasm had appeared in the last two years. The fish was collected and transferred to the Centre for Experimental Fish Pathology of Sicily at the Department of Veterinary Sciences, University of Messina, where it was held in a 200 L tank containing fresh water, at 22°C. To avoid killing the fish, a small sample of tumour tissue was collected by biopsy using a punch of 0.2 cm. The tissue specimens obtained were processed for histological, histochemical and immunohistochemical examination to identify the cell origin. The neoplastic tissue was transversally and longitudinally microdissected and fixed part in 5% buffered formalin solution and part in Bouin’s solution. Processing included dehydration through a graded series of alcoholic solutions, clarification in xylene and finally embedded in paraffin wax at 56°C. Five µm thick tissue sections were treated in Lithium carbonate in 70% alcohol to block the intrinsic peroxidase activity (30 min at Room Temperature); 2) with normal sheep serum to prevent unspecific adherence of serum proteins (30 min at RT); 3) with polyclonal rabbit antibody against S-100 protein (Dako, Glostrup, Denmark, working dilution 1:250) and monoclonal mouse antibody against pancytokeratin (DakoCytomation, Copenhagen, Denmark, w.d. 1:400) at 4 °C for 16 hrs; 4) with sheep anti-rabbit or anti-mouse immunoglobulin antiserum (Behring Institute; w.d. 1:25; 30 min at RT); 5) with mouse anti-horseradish peroxidase-antiperoxidase complexes (Dako Cytomation; w.d. 1:25; 30 min at RT).

For the demonstration of peroxidase activity, the sections were incubated in darkness for 10 min with a 3-3’ diaminobenzidine tetra hydrochloride (DAB; Sigma Chemical Co., St Louis, MO, USA) signal development solution containing 100 mg DAB in 200 ml 0.03% hydrogen peroxide in phosphate-buffered saline (PBS). The nuclear counterstaining was performed by Mayer’s haemalum. 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. Nested PCR targeting a conserved region of Cyprind herpesviruses
(Cy-HVs) DNA polymerase gene, according to the protocol developed by Engelsma and colleagues (2013), was applied on a fragment of the tumor conserved in 70% ethanol. No samples were obtained from lymphoid organs because the fish was still alive, although these organs are appropriate tissues for cyprinid herpesvirus diagnostics. DNA was extracted with the High Pure PCR Template Preparation Kit following manufacturer’s instructions. Koi herpesvirus (KHV) was used as positive control.

The tumour was located in the frontal region of the head. The tissue appeared to arise from epidermis growing outwards, deforming the cranial portion of the head close to and partially covering the left eye (Figure 1). The tumour was 3 cm in diameter, irregularly round, white and firm in consistency. The cut surface was dry and poorly vascularized. Histologically the neoplasia was characterized by a proliferation of monomorphic epithelial cells arranged in lobular structures, surrounded by a thin stroma (Figure 2a). The proliferating tissue tended to grow both outwards and in the dermis mechanically compressing the lower tissues. Within the tumor mass, nests of neoplastic cells showed a centrifuge pattern with evidence of several goblet cells, documented by Alcian Blue pH 2.5-PAS staining (Figure 2b), organized in series or deeply embedded in tumour tissue. Immunohistochemical evaluation showed a strong diffuse cytoplasmic staining of epithelial elements with pan-cytokeratin (Figure 2c). S-100 protein was present only in few scattered nervous structures interspersed in the context of the tumour (Figure 2d). No amplification product of the expected size (339 bp), excluding

![Figure 1. Carassius auratus. The tumour located on the frontal region of the head.](image)
the positive control, was obtained thus confirming the negativity for Cy-HVs of the sample under analysis.

On the basis of anatomo-histopathological, histochemical and immunohistochemical findings a diagnosis of papilloma was made, demonstrating the role of ancillary procedures to achieve a correct differential diagnosis. The finding of this neoplasia in goldfish represents an unusual event in Italy. Morpho-phenotypic features of the present case are similar to those described elsewhere in other fish species i.e. the presence of nests of cellular proliferation as well as goblet cells in the deepest portions of the tumour. One example is a case of stomatopapillomatosis in an European eel (Marino et al., 2010). Moreover, in papillomas described in brown bullheads in New York State (Poulet et al., 1994), multiple neoplasms were more common (89%) than solitary ones (11%) as observed in the current case. The differential diagnosis must consider also carp pox, which is known to be caused by a herpesvirus; this disease is characterized by epidermal hyperplasia easily distinguishable from papilloma because in the latter a participation of the stroma to the neoplastic growth is always present. Immunohistochemical characterization confirmed the presence of cytokeratin as in all epithelial tumours described in human beings and in animals, confirming the need of this antibody marker of the cytoskeleton of the cells with an epithelial embryonal origin to identify the histogenesis of these tumours, although in the present case histology (H&E) and histochemistry (AB-PAS) already strong-
ly supported the diagnosis of papilloma. Our findings are in agreement with data reported in dog papilloma (Kheirandish et al., 2012) as well as in fish papilloma (Marino et al., 2010). The low and scattered expression of S-100, a protein widely demonstrated in fish nervous system (Germanà et al., 2008) and strongly expressed in fish peripheral nerve sheath tumours (PNST) excludes the diagnosis of schwannoma, a tumour commonly reported in goldfish (Marino et al., 2007) and in other fish species (Marino et al., 2008 and 2012). As none of the fish reared in the same tank showed similar tumours, it is unlikely to be caused by a diffuse infective agent as well as by chemical pollutions. Moreover, considering the fish was maintained in not chlorinated water, excludes this as a possible cause.

References


