

DERMAL FIBROMA IN A GOLDFISH : IMMUNOHISTOCHEMISTRY AND POLYMERASE CHAIN REACTION

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ABSTRACT

Naturally occurring case of tumor was identified in dermal of a goldfish. Grossly, this mass was found on the right-dorsal part of the body, white and glossy, firm in consistency, and consisting of variable-sized distinct lobules with narrow hemorrhagic streaks. Histopathologically was composed of wavy spindle-shaped cells with long cytoplasmic processes. They have low cellularity, had elongated or big nuclei with indistinct nucleoli. In the intercellular spaces, mild production of collagen and reticular fibers were found. The neoplastic cells were negative immunoreactivities for vimentin and S100 protein. Polymerase chain reaction/PCRs results showed that the mass was positive for Carp β -actin. Since we used fish genome from a Japanese goldfish as negative control on PCRs it must be consider that the neoplasm is not retrovirus-free. These findings suggest the diagnosis of tumor was a dermal fibroma and may associate with walleye retrovirus (WRV).

Key words: fibroma, histopathology, immunohistochemistry, polymerase chain reaction, goldfish.

INTRODUCTION

Tumors of fibrous connective tissue (fibroblasts) are the most common of the mesenchymal tumors. Fibromas are relatively common tumors affecting a wide variety of different species, and also previously found in goldfish (Constantino *et al.* 1999). This tumor may be loosely attached by tags of fibrous tissue or firm anchored to the tissue of origin. The cells of the typical fibroma are long, spindle-shaped and very densely staining with minimal cytoplasm (Goldschmidt and Hendrick, 2002).

Previous studies reported that environmental and infectious agents are suggested as the causative agents of fibromas, which can often occur at high levels in farmed and wild fishes, but little is known of the precise factors operating in such cases. Predisposing factors such as carcinogenic compounds, viruses, irritans, oncogenes and parasites have all been reported in teleost and should be considered potential sources for tumor induction in tropical fish (Stoskopf, 1993). This study was performed to observe pathomorphology of the mass and clarify relationship between those mass and walleye retrovirus (WRV) infection.

METHODS

Eight-goldfishes had been held together in the freshwater for approximately 15 months when a small mass (approximately 0.6 cm in diameter) firstly noted on the left side of the body of a goldfish. The mass on the goldfish was surgically removed by the veterinarian and then was returned to the display tank. Twelve-months later the mass reappeared at the same location, close to the dorsal fin and the mass became bigger and multinodules (approximately 1 cm in diameter). The fish was removed from display tank and submitted to Laboratory of Veterinary Pathology, Bogor Agriculture University. Euthanasia was performed at the owner's request.

Representative tissues were fixed in 10% buffered neutral formalin and routinely embedded in paraffin. For light microscopic evaluation, the sections were stained with hematoxylin and eosin (HE). And serial sections were stained with Masson trichrome, Silver impregnation (Watanabe), Bodian's and Alcian Blue - Periodic Acid Schiff (AB-PAS). Additional formalin-fixed, paraffin-embedded sections were stained by using streptavidin-biotin complex (SAB) immunoperoxidase method. The primary antibodies employed were the following: mouse anti-vimentin (1:200, DAKO M7020) and rabbit anti - S100 antibody (1:2000, DAKO Z0311).

Frozen tumor was processed by using Polymerase Chain Reaction (PCR) for detection of Carp β -actin and walleye

retrovirus pol gene (WRV pol gene). The PCR was performed using TAKARA Ex Taq HS.

Table 1. Sequences of oligonucleotide primers

Primers	Sequence (5' to 3')	Position
Carp β -actin Fwd	TGGCAATGAGAGGTTTCAGGT	1155 - 1174
Carp β -actin Rev	CTCCTGCTTGCTAATCCACATC	1652 - 1673
WRV pol Fwd	GCTGATGAGGCCGCTAAAAA	4874 - 4893
WRV pol Rev	GTATGAGAAACCCCATGTAT	2491 - 2510

RESULTS AND DISCUSSION

Grossly, a multinodular mass has firm consistency, was found on the right-dorsal part of the body. Examination on cross section revealed a white and glossy mass consisting of variable-sized distinct lobules with narrow hemorrhagic streaks. Some parts of the mass were soft and moist.

Histopathologically, the present neoplasm was multilobular and composed of wavy spindle-shaped cells with long cytoplasmic processes. They have low cellularity, had elongated or big nuclei with indistinct nucleoli. Mitotic figures were infrequently observed. The cytoplasm was scanty and had pale eosinophilic in color. Neoplastic cells formed many wave and "herring bone" pattern, separated by spacious interstitium and had mildly collagen fibers (Masson trichrome). The neoplastic mass contained mild hemorrhages and multifocal variable-sized areas of necrosis. There was mild production of reticular fibers in neoplastic tissue by using Silver impregnation (Watanabe) stain, and the results for Bodian's and AB-PAS were negative. Immunohistochemistry method showed that neoplastic cells were negative immunoreactivities for vimentin and S 100 antibodies.

Neoplastic cells were spindle cells, with elongated nuclei and indistinct nucleoli, produced small amount of collagen and reticular fibers. As with the previous studies, the mass histopatologically was consistent with dermal fibroma. Many fibromas showed frequently positive immunoreactivities for vimentin, and negative for S-100 protein. This case was negative for those antibodies, probably the antibodies were not reactive for fish specimens. Many fish diseases are

suspected of having a viral etiology. Studies of tumor induction by retroviruses and DNA viruses have led to key advances in understanding cell proliferation and oncogenesis. The present results of PCRs were positive reaction for Carp β -actin and negative for WRV pol. In the case of walleye and perch retroviruses, regression represents a unique process wherein the necrotic tumor provides an environment for expression of very high levels of infectious virus (Quackenbush *et al.* 2001).

In this study we prepared fish genome from a Japanese goldfish and used it as negative control. Therefore we cannot conclude that present fibroma in a goldfish is retrovirus-free or cannot detect it with primer sets reported by other researchers. In further studies are needed to clarify tumor in fish by employing specific antibodies which match to fish tissues.

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