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Studies on a New Virus (OMV) from *Oncorhynchus masou*—II. Oncogenic Nature

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Among survivors of experimental infection of herpesvirus OMV which was considered to be a new viral pathogen of salmonids producing hepatic necrosis and significant mortality, more than 60% developed tumors. The perioral maxillary and mandibular region are the most frequent site of tumor formation. In decreasing order of frequency, tumors were also found on the caudal fin, gill-cover, eye, and kidney.

Histopathologically the tumors were composed of abundantly proliferative, well differentiated epithelial cells supported by fine connective tissue stroma.

Although virus particles were not observed in tumor cells, OMV isolation was successful from a tumor tissue sample that appeared necrotic on day 275 postinfection and from primary cultures of a tumor tissue from another fish sampled 296 days postinfection.

From the evidence thus far obtained, OMV is considered to be a new pathogenic and oncogenic salmon virus.

It is the first oncogenic agent to be isolated from fish.

We have previously described that *Oncorhynchus masou* virus (OMV), a new herpesvirus, which had been isolated from ovarian fluids of landlocked masu salmon (*Oncorhynchus masou*) in September 1978, was considered to be a new viral pathogen producing hepatic necrosis and significant mortality in experimentally infected salmon fry (KIMURA *et al.*, 1981).

Some fry survived experimental infection, and among them abnormal masses diagnosed as epidermal tumors have been found on the body surface. The first tumors developed at 130 days postinfection, and the incidence increased with time.

Although tumors of fishes have been described by a number of authors with some speculations for viral etiology (WESSING and BARGEN, 1959, WINQVIST *et al.*, 1968, WALKER, 1969, DAYS, 1976, KIMURA, 1976, SCHWANZ-PFITZNER, 1976, SONSTEGARD, 1976, and KELLY *et al.*, 1980), no experimental evidence has been reported so far.

In this report, we describe the experimental induction of tumors with a newly isolated salmonid herpesvirus, OMV.

Tumor induction with OMV:

Fiftytwo chum salmon (*Oncorhynchus keta*)

fry that survived experimental infection with OMV (at 150 days of age) described previously (KIMURA *et al.*, 1981) were held for further observation. Tumor masses were first recognized on day 130 postinfection. This mass occurred principally about the mouth but also on gill-cover, eye, and caudal fin as shown in Fig. 1. More than 60% of the fish that survived infection with OMV developed tumors until 250 days postinfection. Some fish had tumors in multiple sites and on day 312 one of 52 fish had a renal tumor.

Incidence of tumors:

As shown in Fig. 2, the perioral maxillary and mandibular regions were the most frequent site of tumor formation. In decreasing order of frequency, tumors were also found on the caudal fin, gill-cover, eye, and kidney. Uninfected control fish held under the same conditions showed no tumors, therefore the neoplasms can be attributed to OMV infection.

Histopathology of tumors:

Histologically the mandibular tumors were characterized as papillomas consisting of abnormally proliferating epithelial cells (Fig. 3). Structurally, there were several layers of squamous

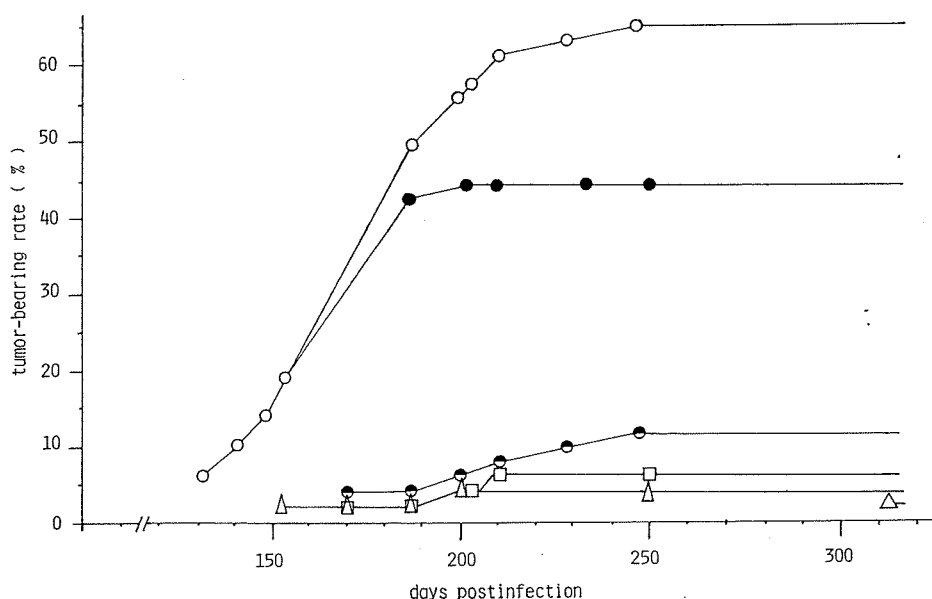


Fig. 1. Incidence of tumor development among chum salmon (*Oncorhynchus keta*) following exposure to OMV.

○—○: total. ●—●: around the mouth. ◐—◐: caudal fin. △—△: eyelid. □—□: gill cover. ▴—▴: kidney.



Fig. 2. Tumor developing around the mouth of a chum salmon fingerling.

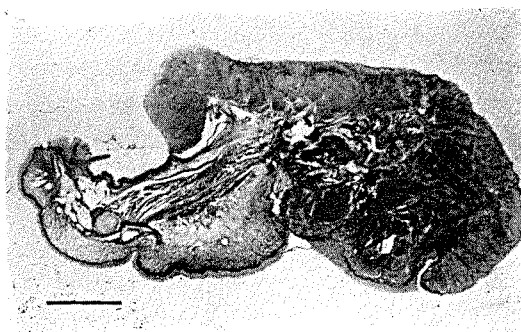


Fig. 3. Transverse section of the jaw of a tumor bearing chum salmon fingerling (— 1 mm).

epithelial cells in papillomatous array and supported by fine connective tissue stroma. Abundant mitotic figures suggested a highly proliferative nature (Fig. 4).

Ocular tumors showed similar characteristics to those the mouth, abnormal growth of epithelial cells occurred in the cornea. Hemorrhage of unknown cause was observed under the connective tissue of cornea.

The opercular tumors also had the same ap-

pearance, and some cases the tumor showed a close attachment to the gill tissue.

The caudal fin tumors had early changes of tumor formation. The normal arrangement of the epithelial cells was disturbed by areas of abnormal growth, although support by fine connective tissue stroma was lacking.

The renal tumor also consisted of epithelial cells. The central area of the tumor showed necrosis, and the normal kidney tissue was displaced (Fig. 5).

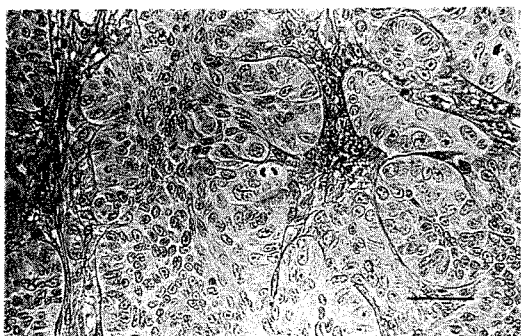


Fig. 4. Histological section of tumor tissue from the jaw of a chum salmon fingerling. High magnification of a tumor tissue section characterized by abnormal proliferation of epithelial cells (— 20 μ).

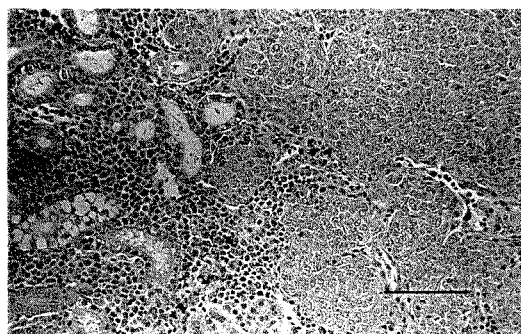


Fig. 5. Histological section of a tumor developing in kidney tissue of a chum salmon fingerling (— 50 μ).

The above findings, suggest that the tumors are malignant.

Electron microscopy:

Electron microscopy revealed that the tumor cells had a typical neoplastic feature of variability in nuclear size, and loose intercellular connection. However, OMV particles were not found in the nuclei or the cytoplasm of the tumor cells (Fig. 6).

Recovery of OMV from tumor-bearing fish:

Virus reisolation procedures were carried out on tumors, liver, kidney, heart, and spleen tissues of 11 tumor bearing fish.

As shown in Table 1, virus was recovered and serologically identified as OMV from a tissue

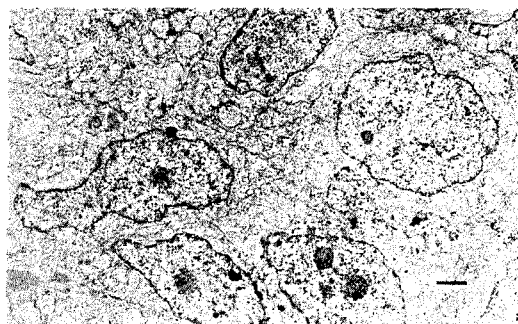


Fig. 6. Electron micrograph of tumor cells. Virus particles were not found in the nuclei or cytoplasm (— 1 μ).

Table 1. The results of virus reisolation from tumor bearing chum salmon (*Oncorhynchus keta*) exposed to OMV

Fish No.	Days after exposure	B.W. (g)	B.L. (cm)	Tumor bearing region	Specimen					
					Liver	Kidney	Spleen	Heart & spleen	Tumor tissue	Primary culture of tumor cell
1.	141	17.8	12.2	premaxilla	—	—	—	—	—	
2.	275	40.0	15.5	& mandible	—	—	—	—	—	
3.	"	60.2	17.5	"	—	—	—	—	—	
4.	"	32.0	16.0	caudal fin	—	—	—	—	—	
5.	"	35.5	15.5	premaxilla	—	—	—	—	+	
6.	"	11.5	11.5	& mandible	—	—	—	—	—	
7.	296	59.7	16.0	"	—	—	—	—	—	+
8.	"	27.4	19.8	"	—	—	—	—	—	
9.	141	19.5	11.8	normal	—	—	—	—	—	
10.	"	24.5	13.8	"	—	—	—	—	—	
11.	"	15.9	11.2	"	—	—	—	—	—	

sample that appeared necrotic on day 275 postinfection. Most of the samples, however, were without evidence of infectious virus. Furthermore, the medium from the primary culture of a tumor tissue from another fish sampled 296 days postinfection also revealed virus. The primary culture showed progressive growth during the first 4 days but then cytopathic changes developed, and virus was recovered.

Neutralizing antibody against OMV in sera of experimental fish:

Serum samples from survivors of experimental infection were assayed for anti-OMV neutralizing activity. As shown in Table 2, sera of survivor fish, that had tumors or that did not, showed higher titers of neutralizing antibody than the uninfected control fish.

Table 2. Neutralization titer* of tumor bearing and non-bearing, OMV infected chum salmon (*Oncorhynchus keta*) sera against OMV

serum	days after exposure	neutralization titer*
tumor bearing fish, 5 fish pool	275	1: 160
tumor bearing fish	296	1: 160
tumor non-bearing fish	"	1: 320
non-infected control fish	296	1: <20
non-infected control fish	"	1: <20

* completely neutralize the 100 TCID₅₀ OMV.

In the following year, these observations were reproducible in repeated experiments using a different lot of chum salmon. Tumor formation was observed in 50 to 60% of the survivors during 110 to 130 days postinfection. This period was the same as in the first experiment. Tumors were also induced in coho salmon (*Oncorhynchus kisutch*) approximately days 110 postinfection (YOSHIMIZU *et al.*, 1980).

These results suggest that OMV is pathogenic for salmonids and it causes not only hepatic necrosis it is also oncogenic in some infected fish.

Suspected viral etiology has been described for certain fish tumors; as cauliflower disease of eels (*Anguilla anguilla*) (DEYS, 1976; SCHWANG-PFITZNER, 1976), lymphosarcoma of pike (*Esox lucius* and *Esox masquinongy*) (SONSTEGARD, 1976; WINQVIST

et al., 1968), renal tumor of guppy (*Lebistes reticulatus*) (WESSING and BARGEN, 1959), and epithelial malignancy and sarcoma of walleye (*Stizostedion vitreum*) (WALKER, 1969; KELLY *et al.*, 1980), however direct evidence is still lacking.

KIMURA (1976) also reported association of some infectious agent with recent outbreaks of oral epithelial tumors in cultured salmonids on Honshu Japan, although pathogenesis remains to be determined.

The present study, however, provides the first and most convincing evidence of viral oncogenesis that tumors developed following infection with a newly isolated herpesvirus of salmonids.

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