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***Oncorhynchus masou* Virus (OMV): Incidence of Tumor Development among Experimentally Infected Representative Salmonid Species**

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Coho (*Oncorhynchus kisutch*), chum (*O. keta*), masu salmon (*O. masou*) and rainbow trout (*Salmo gairdneri*), which survived experimental infection with *Oncorhynchus masou* virus (OMV), were observed for the development of tumors induced by OMV.

Tumors in coho and chum salmon were first observed 120 days post-infection and after 200 days, 35% of the coho and 40 to 60% of the chum salmon were affected. The rate of tumor induction was not influenced by the age of the fish at the time of infection. Tumors of rainbow trout and masu salmon were not present at 200 days post-infection but appeared after 240 and 270 days, and the rate of tumor inducement reached 12% for rainbow trout and almost 100% for masu salmon after 365 days.

Tumors occurred mainly around the mouth, but were also observed on the fins, opercula, body surfaces and corneas of the eyes. Histopathologically, the tumors were composed of abundantly proliferative, well differentiated epithelial cells supported by fine connective tissue stroma. OMV was recovered from the culture medium of one passage of the transplanted tumor cells by primary culture series.

Oncorhynchus masou virus (OMV) is a fish herpesvirus isolated from the ovarian fluid of landlocked masu salmon (KIMURA *et al.*, 1981a) and has a pathogenicity against the fry of masu salmon and several salmonid fish. In particular, masu, chum (*O. keta*) and kokanee salmon (*O. nerka*) usually exhibited high susceptibility with more than 80% of the fry dying within 4 months after infection (KIMURA *et al.*, 1983). Affected fish became dark and occasionally had severe exophthalmia and hemorrhage under the jaw before death. Internally, the kidney was pale and multiple white spots were observed on the liver (TANAKA *et al.*, 1984).

The oncogenic nature of OMV was first noticed in tumors of chum salmon which survived OMV infection at 130 days after infection, and the rate of tumor induction reached about 60% at 250 days post-infection. The most frequent site for tumor formation was above the mouth and, in decreasing frequency, the caudal fin, opercula and corneas of the eyes and one of the 52 fish was found to

have had a renal tumor at 10.5 months after infection (KIMURA *et al.*, 1981b).

This report describes in detail the oncogenicity of OMV using representative salmonid species at different fry stages.

Materials and Methods

Fish

The following fish were observed for the development of tumors induced by OMV; forty coho salmon fry that survived experimental infection with OMV at the age of 1 month; 10, 10, 2, 7, 20 and 13 chum salmon fry which survived OMV infection at the age of 1, 2, 3, 4, 6 and 7 months old respectively; 10 and 28 masu salmon fry infected at 3 and 5 months; and 42 rainbow trout infected at 1.6 months as described previously (KIMURA *et al.*, 1983). Fish were held in running water at 10-15°C and the tumor bearing fish were recorded daily until the end of a 200 day period for coho and chum salmon, and one year for masu salmon

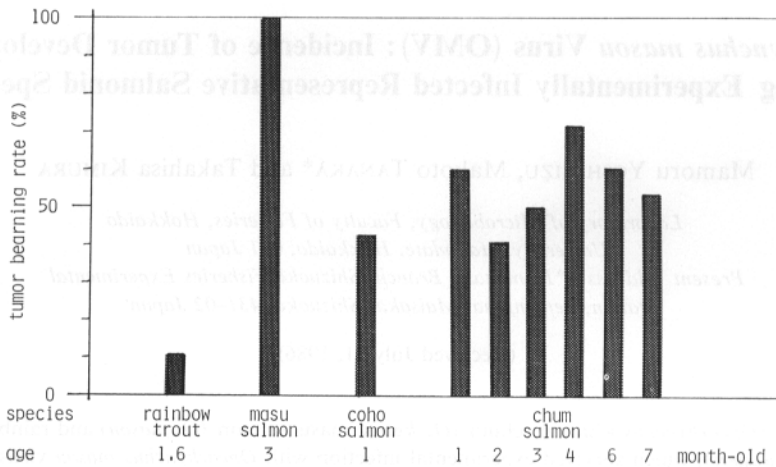


Fig. 1. Comparison of tumor bearing rate in the fry stage of rainbow trout (*Salmo gairdneri*) masu salmon (*Oncorhynchus masou*), coho salmon (*O. kisutch*) and chum salmon (*O. keta*) exposed to OMV.

and rainbow trout.

Isolation of OMV from tumor tissue

Isolation of OMV from tumor tissue was carried out by using the primary culture method. The tumor tissues were removed and disinfected with iodophore (50 ppm, 20 min) and then washed 3 times with Hanks' BSS. After a one night trypsinization with 0.25% trypsin in PBS at 5°C, 3.5×10^5 tumor cells/ml were seeded in a tissue culture flask (Falcon) and incubated with Eagle's MEM containing 20% FBS (Gibco), 100 I.U. penicillin and 100 µg streptomycin (Sigma)/ml. After the transplantation of primary culture cells, the virus inspection of the culture medium was carried out using the RTG-2 cells (WOLF and QUIMBY, 1962).

Histopathology of tumor tissue

Fish that had developed tumors were sampled and the tumor tissues were fixed in Bouin's solution and then transferred into 90% ethanol until processed. Tissues were dehydrated in graded alcohol and embedded in paraffin wax. Sections were cut at 4–6 µm and stained with hematoxylin and eosin stain.

Results and Discussion

Tumor induction with OMV

Tumors in coho salmon were first observed at 120 days post-infection and after 200 days, 35% of fish had become affected. In the chum salmon, tumors appeared at about 120 days post-infection and the rate of tumor inducement reached 40 to 71% after 200 days. The rate of tumor induction was not influenced by the age at which fish were infected. Tumors in rainbow trout and masu salmon were absent at 200 days post-infection but appeared after 240 and 270 days, and the rate of tumor inducement reached 12% for rainbow trout and almost 100% for masu salmon after 365 days (Fig. 1).

A tumor formation site for coho salmon is shown in Fig. 2; the perioral maxillary and mandibular regions being the most frequent site of tumor formation. In decreasing order of frequency, tumors were also found on the caudal fin, gill cover and eye. This inducement pattern of tumor formation site was similar to that of chum salmon as described previously (KIMURA *et al.*, 1981c).

Uninfected control fish held under the same conditions had no tumors and, therefore, the neoplasm can be attributed to OMV infection.

Isolation of OMV from tumor tissues

OMV was not isolated from the filtrated (0.45

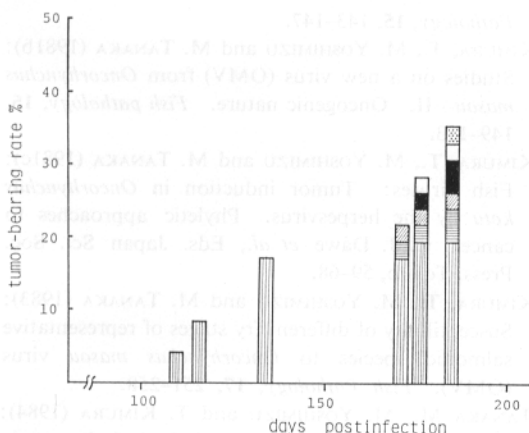


Fig. 2. Incidence of tumor development among coho salmon following exposure to OMV: ■ around the mouse, ▨ gill cover, ▩ body surface, ■ fin, □ around the vent, ▤ eyelid.

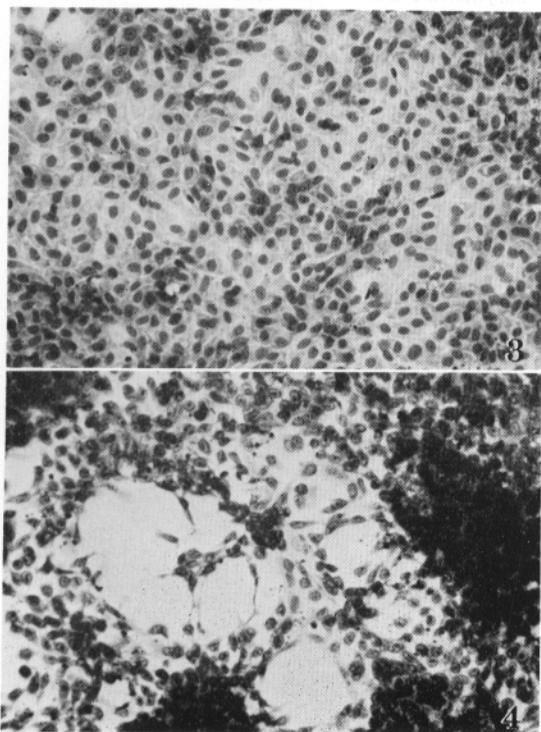


Fig. 3. Cell sheet of primary culture derived from tumor tissue of chum salmon infected with OMV. May-Grünwald Giemsa stain.

Fig. 4. CPE like change appeared on cell sheet after one transplantation of primary culture, May-Grünwald Giemsa stain.

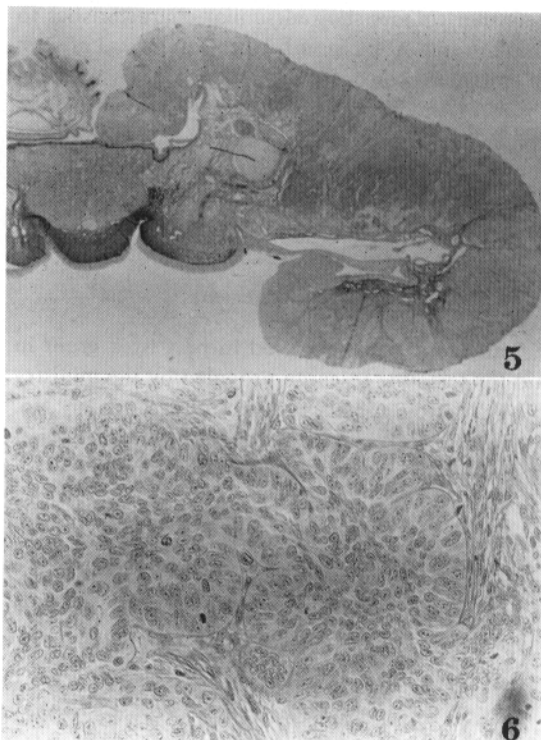


Fig. 5. Section of tumor observed on the jaw of chum salmon survived OMV infection. HE stain.

Fig. 6. High magnification of tumor section observed on the jaw of chum salmon. HE stain.

μm Millipore filter or $0.40 \mu\text{m}$ Nucleopore filter) homogenate of tumor tissue, but was isolated from the culture medium of tumor cells after one passage of primary culture cells. Although tumor cells grew well on primary cultures and did not show CPE-like changes (Fig. 3), the CPE-like changes (Fig. 4) were observed after 3 to 5 days on the transplanted primary culture cells; then, OMV was recovered from the culture medium of these cells.

Histopathology of tumors

Histopathologically the mandibular tumors of coho, masu and rainbow trout were characterized as papillomatous consisting of abnormally proliferating epithelial cells (Fig. 5). Structurally, there were several layers of squamous epithelial cells in papillomatous array and supported by fine connective tissue stroma. Abundant mitotic figures suggested a highly proliferative nature (Fig. 6).

Ocular tumors showed similar characteristics to those found in the mouth where abnormal growth of epithelial cells occurred in the cornea. Hemorrhage of unknown cause was observed under the connective tissue of the cornea. The opeular tumors also had the same appearance in some cases. The details of the histopathological study of these tumors will be published in the near future.

Acknowledgements

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