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HERPESVIRUS INFECTION OF SALMONID FISH

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INTRODUCTION

Herpesvirus infection of salmonid fish was first reported by Wolf and Tailor (1975) in America on isolation of *Herpesvirus salmonis* from ovarian fluids of apparently normal rainbow trout (*Oncorhynchus mikys*). In Japan, nerka virus Towada lake, Akita and Aomori Prefecture (NeVTA) isolated from moribund kokanee salmon (*O.nerka*) in Towada Lake was first reported by Sano (1976). Subsequently, Wolf et al. (1975) suggested a herpesvirus infection among kokanee salmon in Shikotsu lake in Hokkaido, but the virus could not be isolated. In 1978, *Oncorhynchus masou* virus (OMV) was isolated from ovarian fluids of normal appearing adult masu salmon (*O.masou*) at the Otobe Salmon Hatchery, Hokkaido (Kimura et al. 1980a). In 1981, yamame tumor virus (YTV) was isolated from tumor tissue of yamame cultured in Niigata (Sano et al. 1983) and also herpesvirus strain 83 (H-83) was isolated from ovarian fluid of masu salmon cultured in Chitose, Hokkaido (Hayashi et al. 1986). In 1986, steelhead trout virus (SHV) was isolated from anadromous salmonids (Hedrick et al. 1986). Recently, herpesvirus was isolated from tumor tissue, liver and skin lesion of cultured coho salmon (*O.kisutch*) caused major problem in Tohoku district (Sano et al. 1988, Horiuchi et al. 1989, Kimura and Yoshimizu 1991, Kumagai et al.1991).

COMPARISON OF SALMONID HERPESVIRUS BY ANTIGENIC PROPERTIES:

Serologically, *H.salmonis* and SHV isolated in America is confirmed to be the same virus. OMV, NeVTA and YTV isolated in Japan is also confirmed to be the same virus. On the other hand, herpesvirus isolated in America has been distinct from viruses isolated in Japan (Hedrick et al. 1987). Difference between these viruses have been also recognized on the basis of virus induced polypeptide patterns (Kimura et al. 1984). Therefore, herpesvirus isolated in America provisionally designated as salmonid herpesvirus 1 and in Japanese designated as salmonid herpesvirus 2 (Roizman et al. 1981).

Herpesvirus has been recently isolated from coho salmon in Tohoku district. These viruses were all neutralized by anti-OMV serum. Furthermore, herpesvirus strain 83 is serologically distinct from OMV, but these viruses have been confirmed to be the same virus by DNA restriction endonuclease cleavage analysis (Hayashi et al. 1987) (Table 1).

SALMONID HERPESVIRUS 1: *RPESVIRUS SALMONIS*

A 30 to 50 % postspawning loss among broodstock rainbow trout have occurred annually since 1971 at the Winthrop National Fish Hatchery in

Table 1 Herpesvirus isolated from salmonid fish

Name	Species	Place	Reference
H.salmonis	Rainbow trout (*O. mikys*)	America	Wolf and Tailor 1975
NeVTA	Kokanee salmon (*O. nerka*)	Japan	Sano 1976
OMV	Masu salmon (*O. masou*)	Japan	Kimura et al. 1980
YTV	Yamame (*O. masou*)	Japan	Sano et al. 1983
SHV	Steelhead trout(*O. mikys*)	America	Hedrick et al. 1986
H-83	Masu salmon (*O. masou*)	Japan	Hayashi et al. 1986
CSTV	Coho salmon (*O. kisutch*)	Japan	Sano et al. 1983
OKV	Coho salmon (*O. kisutch*)	Japan	Horiuchi et al. 1989
COTV	Coho salmon (*O. kisutch*)	Japan	Kimura & Yoshimizu 1991
CSLV	Coho salmon (*O. kisutch*)	Japan	Kumagai et al. 1991

the state of Washington. A herpesvirus, named *Herpesvirus salmonis*, was isolated from post-spawning rainbow trout. *H.salmonis* was classified to a member of herpesvirus family from the biophysical and biochemical properties (Wolf et al. 1978). Hexagonal nucleocapsids in nuclei had a diameter of 90 nm and enveloped forms measured about 150 nm.

By artificial infection, rainbow trout fry and fingerlings are the only known susceptible species (Wolf et al. 1975). Fingerling atlantic salmon (*Salmo salar*), fingerling brown trout (*S.trutta*) and fingerling brook trout (*Salvelinus fontinalis*) injected intraperitoneally with virus were found to be refractory. Histopathologically, visceral and respiratory organs and heart showed major pathological changes and pancreatic syncytia were judged to be pathognomonic. Kidneys were prime targets for the virus (Wolf and Smith 1981).

SALMONID HERPESVIRUS 2: OMV

Biophysical and Biochemical Properties of OMV

OMV was initially isolated in September 1978 from ovarian fluids of normally appearing mature masu salmon cultured in Otobe Salmon Hatchery, Hokkaido. Although there were no signs of virus infection among adult salmon, population history of the hatchery showed a pattern of low survival among the progeny fry. Cytopathic effect (CPE) distinct from that caused by IPNV or IHNV was observed in 4 samples inoculated on to RTG-2 cell.

At a near-optimal incubation of 15°C, OMV shows distinctive CPE within 5 to 7 days. The CPE is characterized by rounded cells followed by syncytium formation and eventual lysis. Cell lines all derived from salmonid fish tissues including RTG-2 are susceptible to OMV. But, Cell lines derived from non-salmonid species such as BB, FHM, SBK, EPC, EO-2 and EK-1 are refractory.

Characteristics of the OMV are summarized in Table 2. OMV is heat-, ether-, and acid-labile, and does not hemagglutinate human O cells. It is completely inactivated by ultraviolet irradiation. In the presence of the pyrimidine analogue, 5-iododeoxyuridine, replication is inhibited, indicating that the virus has a DNA genome.

Electron microscopy of OMV infected cells reveals the intranuclear hexagonal capsids with a diameter of 115 nm. Abundant budding enveloped virions, 200 x 240 nm in diameter, were also observed on the surface and

inside cytoplasmic vesicles. The calculated number of capsomeres of negatively stained virions is 162.

These features of OMV conform with the characteristics of the herpesvirus group (Kimura et al. 1981a).

Table 2 Characteristics of *Oncorhynchus masou* virus

IUdR, 50 micro-gr/ml	Inhibit
PH 3.0 (acetate HCl buffer) 15°C, 3hrs.	Inactivate
U.V. irradiation 3,000 micro-W sec/cm^2	inactivate
Ether, 5°C, 18 hrs.	Inactivate
Heat 50°C, 30 min.	Inactivate
60°C, 30 min.	Inactivate
Released capsid (negative stained)	97+10nm
Capsid intra-nucleus RTG-2 cell	115+7nm
Capsid intra-envelope	115+16nm
Enveloped vilion	240+23 x 200+24nm
Calculated number of capsomer	162

Pathogenicity

Susceptibility of several salmonid fry to OMV has been studied experimentally by immersion in water containing 100 $TCID_{50}$/ml of OMV at 10°C for 1 h. Compared the five different salmonid fry, at the age of 1-month, kokanee salmon exhibited the greatest sensitivity, and 100 % of them died. Masu salmon and chum salmon also exhibited high sensitivity at 87 % and 83 %, respectively. Coho salmon and rainbow trout were shown to be less sensitive to OMV infection at 39 % and 29 % mortality, respectively. Thus the host range of OMV should be wide in salmonid species (Kimura et al 1983a).

Several age groups of one hundred fry of chum salmon, 0, 1, 2, 3, 4, 5, 6, and 7 month-old, were immersed under the same conditions. The cumulative mortality of just hatching chum salmon, observed in ensuing 4 months, was 35 %, but between 1-month and 5-month-old fry, it was more than 80 %. In particular, at 3-month-old, the fry exhibited 98 % mortality, which should be the greatest sensitivity. At 6 and 7 months, the fry's susceptibility was reduced and only 7 % and 2 % fish had succumbed. There were no deaths among 8-month-old fingerings which were immersed in a suspension of virus and additionally injected intraperitoneally with 200 $TCID_{50}$/fish (Kimura et al 1983a).

On the other hand, 1-month-old masu salmon fry were the most sensitive and the cumulative mortality reached 87 %. In 3- to 5-month-old fry, cumulative mortality decreased from 65 to 24 % (Kimura et al. 1983a).

Fish-to-fish transmission was demonstrated by holding 5-month-old fry with fry infected by immersion; the resulting mortality was similar to that observed as a result of original infection by immersion (Kimura et al. 1981a).

Disease Signs

Affected fish are anoretic and exophthalmic, they show petechiae on body surface, especially beneath the lower jaw. Internally, livers are mottled with white spots, and in advanced cases the whole organ may become pearly white. Often the spleen is found to be swollen and the digestive tract devoid of food (Kimura et al. 1981a, 1983a).

Histopathology

With respect to one-month-old infected masu salmon, necrosis of haematopoietic tissue in the kidney was observed in all moribund specimens. The kidney was considered to be the principal target organ for the virus as judged by the severity of histopathological changes. Necrosis of epithelial cells of the mouth, jaw, operculum and head were observed in early stage of infected moribund specimens while partial necrosis of the liver, spleen and pancreas was found in later stage of moribund specimens from this group (Table 3) (Tanaka et al. 1984).

In the infected 3-month-old masu salmon, the kidney hematopoietic tissue of nine early moribund specimens were necrotic. In six specimens of long incubation, hyperplasia of the hematopoietic cells was also observed simultaneously. Thus, the kidney of 3-month-old infected masu salmon, gradually become resistant to OMV infection. On the other hand, histopathological foci in the liver tended to become gradually more severe with longer incubation period. For this reason, it was considered that the principal target organ for OMV changed from the kidney to the liver (Table 4) (Tanaka et al. 1984).

Table 3. Results of histopathological observation of one-month-old masu salmon infected with OMV

Fish No.	8	12	22	27	31	32	41	48	51	58	61
Days after infe.	31	39	46	47	48	48	51	53	54	56	58
Kidney	+	+	+	+	+	+	+	+	+	+	+
Epithelial tissue	+	+	+	+	−	+	−	−	−	−	−
Liver	−	−	−	+	−	+	−	−	−	−	−
Stomach	−	−	+	+	−	+	−	+	+	+	+
Spleen	−	−	−	−	−	−	+	+	/	+	/
Pancreas	−	−	−	−	−	−	−	+	−	−	+
Heart	−	−	−	−	−	−	−	−	−	+	−

cf + : Degeneration was observed in the tissue

Table 4. Results of histopathological observation of three-month-old masu salmon infected with OMV

Fish No.	13	27	29	32	35	37	38	46	47	57	59
Days after infe.	30	38	39	41	45	46	46	52	52	59	62
Kidney	+	+	+	+	+	+	+*	+*	+*	+*	+*
Liver	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	/	/	/
Pancreas	−	+	−	−	+	−	−	−	−	−	−
Brain	+	+	+	+	+	+	+	+	+	+	+

cf + : Degeneration was observed in the tissue
* : Hyperplasia of the hematopoietic cells was observed

Liver section from 3-month-old chum salmon, showed marked histopathological changes. These changes were multiple foci of severe necrosis and syncytia formation. Partial necrosis was present in the spleen sections, and cardiac muscle was edematous in some fish (Kimura et al. 1981a).

Electron micrographs of hepatocytes of chum salmon infected with OMV revealed virus replication occurs in the nucleus of the cells. Nuclei of the infected cell become marginated with subsequent aggregation of chromatin. The matured virus particles were observed in the vesicle and around the cell membrane, and enveloped virus particles were seen in the focus of the hepatocyte (Tanaka et al. 1987).

Tumor Induction by Clonic Infection

Tumors in chum and coho salmons were first observed at 120 days post-infection and after 200 days 40 to 60 % of the chum and 35 % of the coho salmon were found to be 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 have not been present at 200 days post-infection but appeared after 240 and 270 days, and the rate of tumor induction reached 12 % for rainbow trout and almost 100 % for masu salmon after 365 days (Yoshimizu et al 1987).

Tumors occurred mainly around the mouth, maxillary and mandibular regions, in decreasing order of frequency, tumors are also found on the caudal fin, gill-cover, body surface, corneas of the eye, and in the kidney. The reisolation of OMV from tumor tissues was succeeded by the primary culture of tumor cells (Kimura et al. 1981b,c, Yoshimizu et al. 1987).

Sera of survivor of fish, that had with or without tumors showed higher titer of neutralizing antibody than the uninfected control fish (Kimura et al. 1981b,c).

Histopathology of Tumor Tissues

Structurally, the neoplasms are characterized as papillomatous consisting of abnormally proliferating epithelial cells. These are several layers of squamous epithelial cells in papillomatous array and supported by fine connective tissue stroma. Abundant mitotic figures suggested a highly proliferative nature. Tumors appeared on caudal fin, gill-cover, body surface, corneas of the eye, and kidney showed similar characteristics to those of the mouth (Kimura et al. 1981b, Yoshimizu et al. 1987).

Electron microscopy revealed that the tumor cells had a typical neoplastic feature of variability in nuclear size, and loose intercellular connection. However, OMV particles have not been found in the nuclei or the cytoplasm of the tumor cells (Kimura et al. 1981b,c, Yoshimizu et al 1987).

Distribution of OMV

From september 1976 to November 1987, 11,095 females of 6 species and 155 males of 2 species of mature salmon were collected to determine the incidence of OMV in northern Japan. With the exception of one hatchery, herpesviruses were isolated from fish at all sites where at least 60 fish could be sampled. All 162 isolates from this survey were neutralized with anti-OMV rabbit serum (Yoshimizu et al. 1989).

Control

In vitro, ACV (acyclovir) and BVdU ((E)-5-(2-bromovinyl)-2'-deoxyuridine) showed high efficacy against the OMV (Kimura et al. 1983b, 1988). In vivo, daily immersion of chum salmon into ACV solution reduced the mortality of the infected fish. Oral administration of the drug did not affect the survival of the chum salmon. On the contrary, the group administered with IUdR by the oral route showed a higher

survival than the ACV-administered group. Daily immersion of infected fish into ACV solution considerably suppressed the development of tumors induced by OMV (Kimura et al. 1983c).

OMV completely inactivated by UV irradiation with 3.0×10^3 micro-W·sec/cm^2 (Yoshimizu et al. 1986).

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