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DETECTION OF VIRAL DNA POLYMERASE ACTIVITY IN SALMON TUMOUR TISSUE INDUCED BY HERPES VIRUS, ONCORHYNCHUS MASOU VIRUS*

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Summary. - DNA polymerase activities were surveyed in tumour tissue and normal tissue of cherry salmon (*Oncorhynchus masou*). High activity of DNA polymerase α was detected in the tumour tissue but not in the normal tissue. This indicates that the tumour cells replicate prosperously. Viral DNA polymerase activity was detected only in the tumour tissue, indicating that *Oncorhynchus masou* virus (OMV) DNA should replicate there. DNA polymerase β activity was of same level in both tissues. This is the first evidence that herpesvirus DNA polymerase was detected in tumour tissue in association with herpesvirus.

Key words: salmon herpes virus; OMV; tumour; DNA polymerase

DNA polymerase α plays main role in DNA replication in eukaryotic cells (Kornberg, 1980), and its activity in tumour cells has been known to increase (Baril *et al.*, 1973). On the other hand, it has been reported that herpes viruses induce viral specific DNA polymerase in the infected cells (Mar and Huang, 1979). We have reported that salmon herpes virus *Oncorhynchus masou* virus (OMV) induces a heatlabile unique DNA polymerase in the infected culture cells (Suzuki *et al.*, 1986). It is known that OMV induces epithelial tumour in the infected salmonids (Kimura *et al.*, 1981). Infectious virus was isolated from the tumour (Kimura *et al.*, 1981), indicating that the virus replicates there. However, this has still not been proved by biochemical methods. We herein surveyed DNA polymerase activities in the tumour tissue beared in cherry salmon (*Oncorhynchus masou*) lower jaw as compared with normal tissue.

The tumour tissue occuring in cherry salmon lower jaws (0.34 g wet weight) was homogenized with sonication in 50 mmol/l Tris-HCl buffer (pH 7.5) containing 200 mmol/l KCl, 1 mmol/l EDTA, 10 mmol/l 2-mercaptoethanol,

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0.5 mmol/l phenylmethylsulfonylfluoride, 20 % (v/v) glycerol and 0.5 % (v/v) Triton X-100. The homogenate was centrifuged at 10 000 rev/min (Tomy TMS-1 rotor) for 30 min. Then the supernatant was dialyzed and applied to phosphocellulose column chromatography. These conditions were the same as in the previous report (Suzuki *et al.*, 1986). DNA polymerases α, β and OMV-polymerase were separated on this column.

Enzyme assays were performed as described previously (Suzuki *et al.*, 1987). The assay systems for each polymerase were specific and could identify all polymerases. The assay mixture for DNA polymerase α contained 50 mmol/l Tris-HCl (pH 7.8), 0.4 mmol/l dithiothreitol (DTT), 4 mmol/l $MgCl_2$, 70 μmol/l each dATP, dGTP, dCTP, 7 μmol/l [^{3}H]dTTP (0.13 kBq/nmol) and 2.5 μg activated salmon DNA. For the assay of OMV polymerase, 100 mmol/l $(NH_4)_2SO_4$ was added to the mixture for DNA polymerase α. The assay mixture for DNA polymerase β contained 50 mmol/l Glycine-KOH (pH 9.5), 0.4 mmol/l DTT, 80 mmol/l KCl, 1 μg poly(rA), 1 μg oligo(dT) and 7 μmol/l [^{3}H] dTTP, and in the case of DNA polymerase γ, the mixture contained 50 mmol/l Tris-HCl (pH 8.5), 0.4 mmol/l DTT, 80 mmol/l KCl, 20 μg poly(rA), 1 μg oligo(dT), 20 mmol/l KPi (pH 8.5) and 70 μmol/l [^{3}H]dTTP. Incubation was performed at 25 °C for 30 min for all polymerases.

Fig. 1 shows the activity of each polymerase obtained from the phosphocellulose column fraction. In the tumour tissue, high activity of DNA polymerase α was observed (Fig. 1A) suggesting the rapid replication of the tissue. This fact

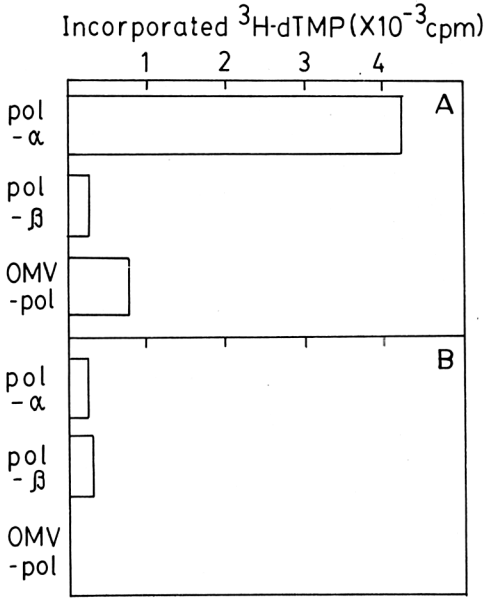

Fig. 1
DNA polymerase activities in tumour tissue (A) and normal tissue (B)
DNA polymerase α (pol-α), β (pol-β) and OMV-polymerase (OMV-pol) activities were assayed by the method reported previously (Suzuki *et al.*, 1987).

indicates that OMV-induced tumour is rapidly growing, which property confirmed our previous report describing microscopic observations (Yoshimizu *et al.*, 1987). The jaw tissue isolated from normal fish did not have such a high activity of DNA polymerase α (Fig. 1B). DNA polymerase β activity, corresponding to DNA repair, was of the same level in both tissues. DNA polymerase γ activity was not detected in both tissues.

Noteworthy observation was that the OMV-induced DNA polymerase was detected in the tumour tissue (Fig. 1A). This suggests that the OMV DNA replicates in tumour tissue. Some herpesviruses have been doubted to be causative agents of cancer (Druff and Rapp, 1971; Rabin, 1985). However, there are no reports on the occurence of viral DNA polymerase activity in solid tumour tissues. Our results shown here are the first evidence suggesting the replication of viral genome in tumour. This viral polymerase activity was not detected thus far in the normal tissue (Fig. 1B).

Although it has been reported that OMV induces epithelial tumours in salmonid fishes (Kimura *et at.*, 1981), the mechanism of the tumour developing is still unknown. The combination of the salmon herpesvirus OMV and host salmonid fishes should be a good model system for the research of herpesvirus-related cancer. As a next step to clarify the tumour developing mechanism, it is desirable to examine the existence form and replication kinetics of the OMV DNA in the tumour tissues.

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