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Research Note

鯉魚鰭的初級細胞培養及其對病毒的感受性

Primary Monolayer Culture of Common Carp (*Cyprinus carpio*) Fin and Its Susceptibility to Fish Viruses

陳俊宏・陳秀男・郭光雄

Jaun-Horng Chen, Shiu-Nan Chen and Guang-Hsiung Kou

The present study aims to describe an establishment of common carp (*Cyprinus carpio*) fin cell culture and to detect the susceptibility of these cell cultures against fish viruses.

Prior to the experiment, common carp weigh approximately 300 gm was dipped into 500 ppm chlorine water for 30 minutes, wiped with cotton saturated with 70% ethyl alcohol for three times and wash thoroughly with sterile distilled water. The tail fin of the experimental fish was then removed using sterile scissors and forceps. For culture experiment, tissue explant technique was employed. The removed tissues was minced with sterile scissors and suspended in phosphate buffered saline (PBS). The small fragments of tissues were then centrifuged and transferred into a 25 cm^2 Falcon flask containing approximately 10 ml of growth medium L-15 (GM L-15) and incubated at a temperature of 31 ± 1°C. GM L-15 is Leibovitz's L-15 culture medium supplemented with 20% foetal calf serum, 50 units/ml penicillin, 50 μg/ml streptomycin and 2.5 μg/ml fungizone. All the culture medium and supplements were obtained from Flow Laboratories, Australia. The cell monolayers were subcultured at a 1:2 split ratio by dispersal with trypsin-EDTA prepared in PBS as described previously (Chen *et al.*, 1981). The cells at a passage level of three were used to detect their ability to support the multiplication of viruses including EVE, EVA, EVEX and IPN. In this experiment, Leighton tubes containing monolayer of common carp fin cells were inoculated with EVE, EVA, EVEX or IPN at a concentration of 10^3 $TCID_{50}$ respectively. For Comparison, the similar concentration of each virus was also inoculated into RTG-2 (Wolf and Guimby, 1962) and EO-2 (Chen and Kou, 1981) cell lines. All the tested cultures were incubated at 20±1°C. The tubes were observed daily at interval of 10 days after inoculation of viruses and the appearance of cytopathic effect (CPE) was recorded.

The results showed that proliferation of the common carp fin cells occurred on the flask surface following 24-48 hours of cultivation at 31±1°C. Confluent cell sheet was obtained in 7-10 days after inoculation of tissue fragments in GM L-15 at 31±1°C. When the confluent cells were cultured at a split ratio of 1:2, it took approximately 5-7 days to become a confluent cell sheet. This result demonstrated that the fin cells of common carp proliferated in GM L-15

* Department of Zoology, National Taiwan University, Taipei Taiwan, Republic of China. 國立臺灣大學動物系。

slowly at 31±1°C. In contract, the eel ovary or kidney cells proliferated very rapidly under the identical culture conditions.

In the primary culture of common carp fin cells only epithelial-like cells were observed. Each cell is characterized by a polygonal cell body containing a centrally-located ovoid-shaped nucleus surrounded by abundant cytoplasm and by a low nuclear-cytoplasmic ratio (Figs. 1-2). Multinucleated cells were observed in all cultures (Figs. 1-2 see arrows).

The susceptibility of EO-2, RTG-2 and common carp fin cells to fish viruses including EVE, EVEX, EVA and IPN are show in Table 1. The results show, that common carp fin

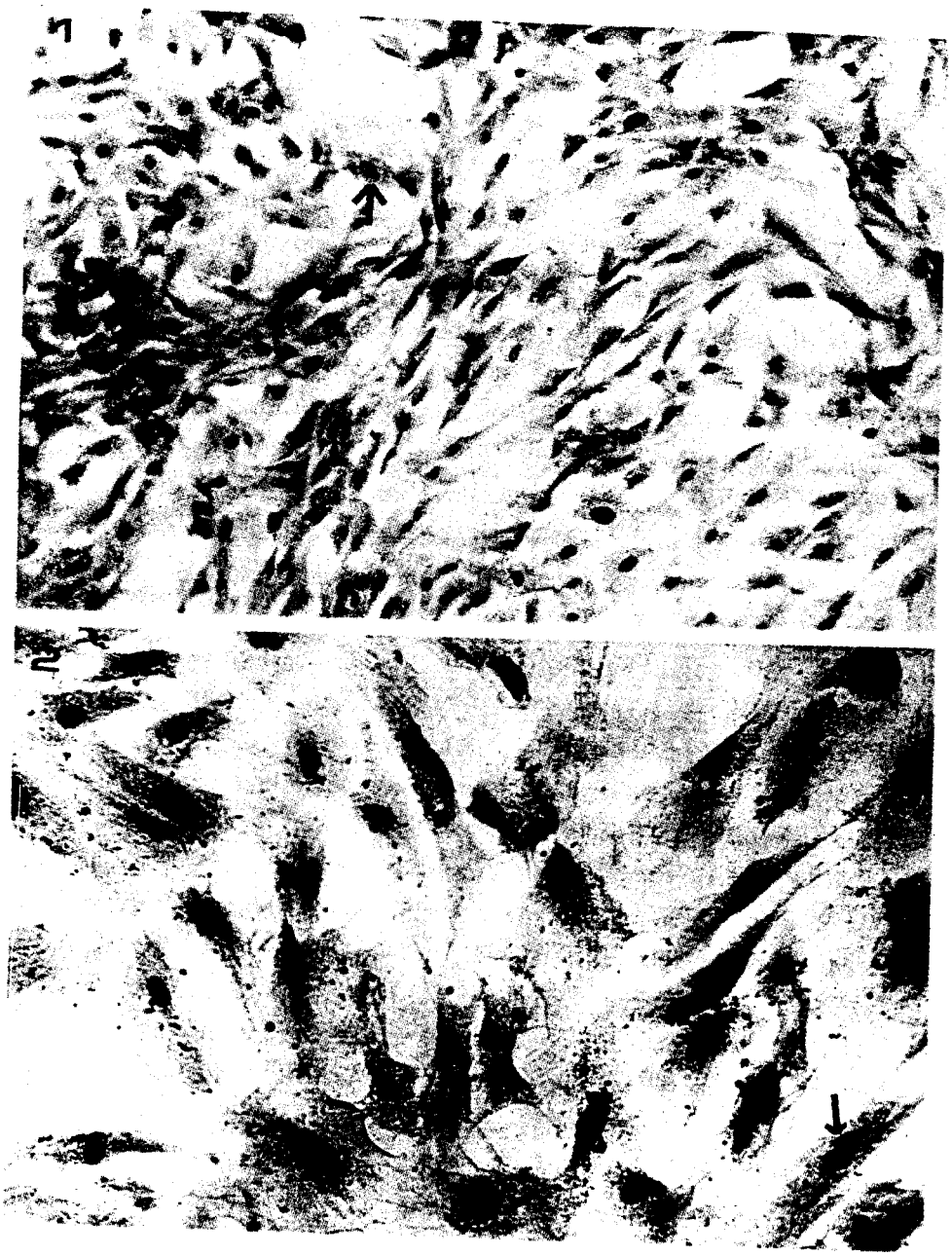

Fig. 1-2. The third subculture of common carp (*Cyprinus carpio*) fin cells, Fig. 1, ×100 Fig. 2, ×200. The arrows in Figs. 1 & 2 show multinucleated epithelial-like cells. Note the polygonal epithelial-like cells with ovoid-shaped nuclei.

Table 1. The susceptibility of common carp (*Cyprinus carpio*) fin, EO-2 and RTG-2 cells to EVE, EVEX, EVA and IPN.

cell culture	viruses			
	EVA	EVE	EVEX	IPN
common carp fin	−	−	−	−
EO-2	+	+	+	+
RTG-2	+	+	+	+

−: No cytopathic effect (CPE) occurred within 10 days' observation at an inoculation concentration of 10^3 $TCID_{50}$ for each virus.

+: Cytopathic effect (CPE) occurred within 5 days' observation at an inoculation concentration of 10^3 $TCID_{50}$ for each virus.

CPE was observed using IM Olympus inverted microscope and Olympus Venox microscope following the specimens had been stained by Giemsa solution.

cells were not susceptible to the tested viruses. On the contrary, either RTG-2 or EO-2 cells were demonstrated to be susceptible to the EVE, EVEX, EVA and IPN. The present results probably suggest that the fish cells originated from internal tissues are more susceptible to fish viruses than those obtained from the epithelial tissues of fish body surface. Experiments are in progress which, it is hoped, will confirm this problem.

中 文 摘 要

本研究目的在於培養鯉魚的鰭細胞，並探討這些細胞對病毒的感受性。細胞經過消毒滅菌後，於 31±1°C 之溫度下，以含有 20%牛胚胎血清的 L-15 培養液來培養，培養細胞之第三代則接種魚類病毒 EVE, EVEX, EVA 和 IPN，而後連續 10 天的觀察這些細胞的病理反應 (cytopathic effect) 之出現情形。實驗結果顯示，培養之鯉魚鰭細胞皆爲多角形之表皮樣細胞，並可觀察出多核之細胞，但這些細胞於 31±1°C 之溫度下在 GML-15 內之生長速率緩慢。本實驗並證實鯉魚鰭細胞對上述四種病毒皆無感受性。

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