農發會漁業特刊第八號、魚病研究專集(四)
CAPD Fisheries Series No. 8, Fish Disease Research (IV), 1-7, 1982, 1

日本鰻魚之腎臟及卵巢細胞株的雙向擴散及免疫電泳研究

Immunodiffusion and Immunoelectrophoresis Studies of Cell Lines Derived from Japanese Eel (*Anguilla japonica*) Ovary and Kidney

陳 秀 男・郭 光 雄

Shiu-Nan Chen and Guang-Hsiung Kou*

Abstract

The application of immunodiffusion and immunoelectrophoresis for distinguishing fish cell lines was evaluated in the present study. The results showed that eel cell lines derived from kidney and ovary tissues revealed cross-reactive precipitin lines with either heterogeneous or homogeneous cell lines. But, the number of precipitin lines resolved in the immunoelectrophoresis plates could be used as criteria for the identification of eel cell lines at a familic level.

By using immunodiffusion and immunoelectrophoresis techniques common cross-reactive antigens between eel cell lines and homogeneous tissues were consistently observed, but they were absent in the heterogeneous systems.

Introduction

Up to the present more than 60 fish cell lines have been established and they were derived from various tissues of approximately 36 species of fish (Wolf and Mann, 1980). Concurrent with the increased number of available lines, there is an urgently need for criteria by which either the cell origin can be identified or one line can be distinguished from the others.

It was demonstrated that cell morphology, growth characteristics, karyologic pattern could be used as parameters for the characterization of animal cell lines from different taxonomic orders. Moreover, immunologic and enzymatic techniques were also suggested to be useful in the identification of cell lines derived from different orders.

Although several mammalian or invertebrate cell lines have been reported to be contaminated by extraneous cells (Fogh, 1973; Greene and Charney, 1971; Greene *et al.*, 1972), Aldridge and Knudson (1980) could distinguish five lepidopteran cell lines by using immunoelectrophoresis. These results may reflect that immunoelectrophoresis is useful in the identification of cell line with different origin.

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

In the present study, the valuation of immunodiffusion and immunoelectrophoresis for the characterization of eel ovary and kidney cell lines (Chen and Kou, 1981; Chen *et al.*, 1982) was detected.

Materials and Methods

Cell Lines

Four fish cell lines including EK-1, EO-2, EPC and RTG-2 and one mammalian cell line, HeLa were used in the present study. All the fish cell lines were cultured in Leibovitz's L-15 medium supplemented with 10% foetal calf serum, 400 Units/ml penicillin, 400 μg/ml streptomycin and 20 μg/ml fungizone. HeLa cells were maintained in Eagle's minimal essential medium (MEM) supplemented with the identical substances as described above. All the tissue culture media and supplements were obtained from GIBCO, New York, USA.

Preparation of Immunizing Antigens

The cell lines grown on 75 cm^2 Falcon flask surface were harvested by centrifugation and followed by washing the cells several times with Hank's balanced salt solution (HBSS). The cell pellet was then re-washed with HBSS for four times to eradicate the substances in the culture media. The final pellet was suspended in a little amount of distilled water and homogenized with 0.5 cc Bellco glass homogenizer. The supernatant was then stored in deep freezer (—70°C) until experimental uses.

Preparation of Antisera

Antisera against EK-1 and EO-2 cell antigens were prepared in two rabbits weighing 2-3 kg. Each rabbit received four weekly subcutaneous inoculation with antigens in an amount of 5, 7.5, 10, 15 mg protein respectively. Prior to the immunization, each antigen was homogenized with complete Freund's adjuvant (Difco) in a ratio of 1:1 (v/v). At the seventh day after the final booster injection, the immunized rabbits were bled by cutting carotid artery and the blood were collected. Antisera were then obtained by centrifugation of the blood at 6,000×g for 10 minutes. The complete Freund's adjuvant was also injected into a rabbit for 4 times weekly. Antisera obtained from this rabbit were used as control.

Preparation of Test Antigen

Cultured cell line and tissue extract antigens were used in the present study.

For the preparation of cell line antigens, the cell lines described above were grown on the surface of 75 cm^2 Falcon flasks and cells were harvested and washed four times in HBSS. The final pellet was extracted using the sucrose-acetone extraction procedures of Clarke and Casals (1958).

Tissue extracts were obtained by homogenizing of tissues from ovary, kidney, spleen and heart of Japanese eel or Common carp respectively. Prior to the homogenization, the individual tissue removed from the fish was washed five times to eradicate the blood. The tissue was then homogenized with 15 cc Ballco homogenizer. Protein concentrations of the preparation were estimated by using the Folin-phenol method (Lowry *et al.*, 1951).

Immunodiffusion and Immunoelectrophoresis

1.2% Agarose (w/v) in veronal buffer (diethylbarbituric acid, 1.4 gm; sodium diethylbarbiturate, 5.0 gm; sodium chloride, 1.0 gm; distilled water 1 liter), pH 8.4, was used for both immunodiffusion and immunoelectrophoresis. Sodium azide was added into the agarose at a final concentration of 0.02% (w/v) to prevent microbal growth.

In immunodiffusion tests, a circular pattern of walls around central well were made on 8×8 cm agar slides, the center-to-center distance from the wells being 1 cm. Each well was 4 mm in diameter. The central wells were filled with antisera and peripheral wells were filled with antigens.

Immunoelectrophoresis was also performed on 8×8 cm agar slides and serum trough (2× 67 mm) was kept 4 mm, edge to edge. The experiment was then carried out at approximately 10°C under 10 mA/Plate constant current.

For both experiments 500 μg protein of antigen was added to each well and antisera were used undiluted.

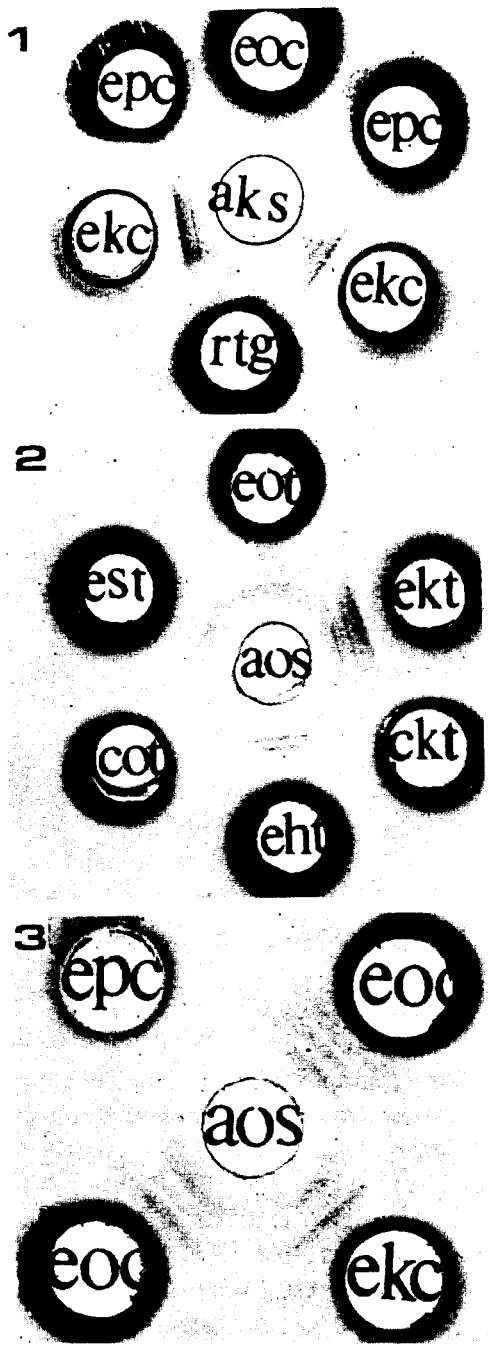
The immunodiffusion and immunoelectrophoresis plates were incubated at 37°C for 48 hours and prepared for staining by washing the plates in three changes of normal saline with a final rinse in distilled water. The agarose was then air dried by covering of filter paper to the plates, stained in 0.1% (w/v) Amindo Black 10 B in 5% (v/v) acetic acid and destained in 7% (v/v) acetic acid.

Results

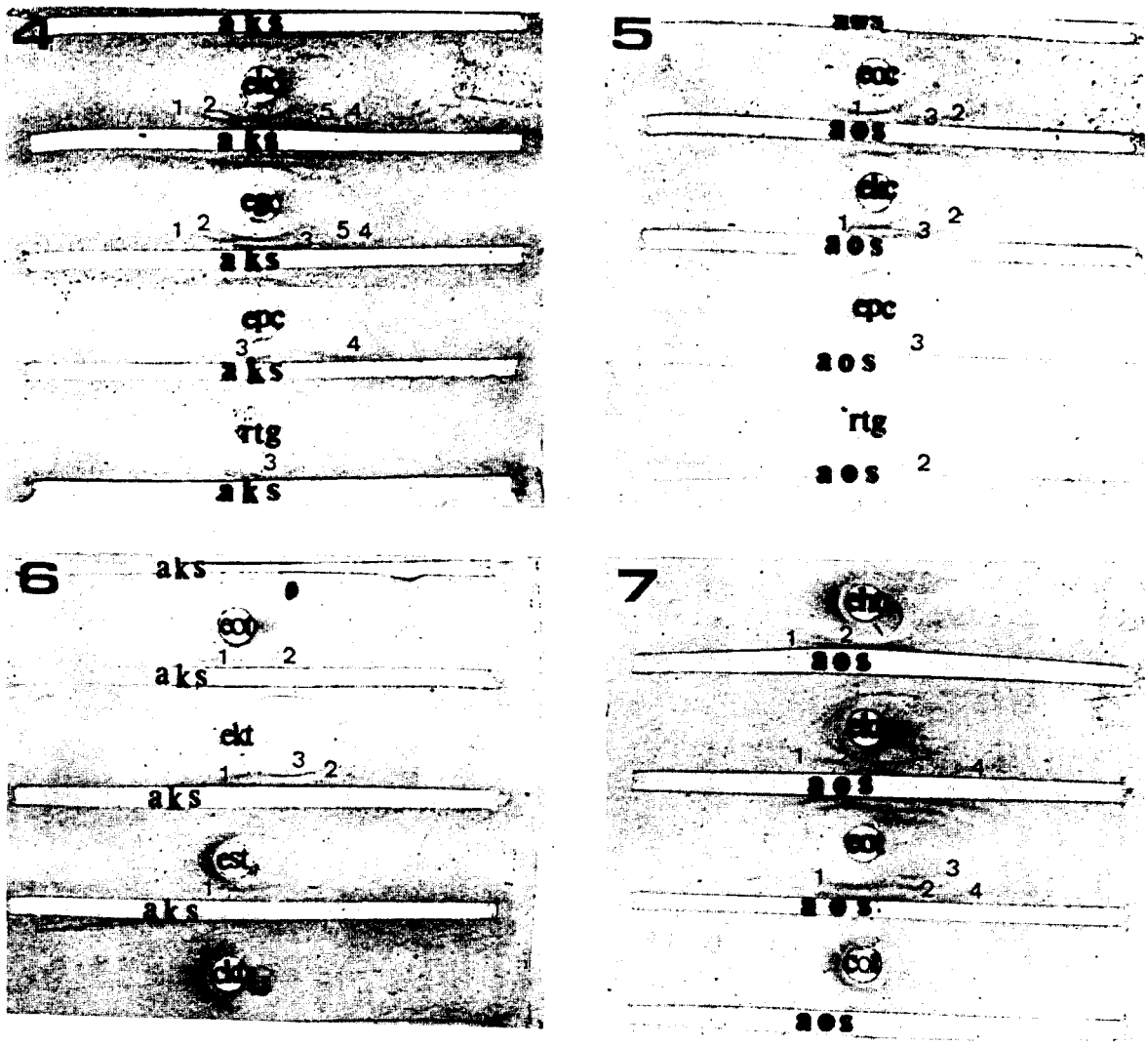
Using immunodiffusion and immunoelectrophoresis no precipitin line was observed between anti-Freund's adjuvant serum and EK-1 or EO-2 cell line antigen. Similarly, the negative result was also obtained when anti-sera against EK-1 and EO-2 cells reacted with mammalian cell line, HeLa.

The immunodiffusion demonstrated that anti-EK-1 and anti-EO-2 sera reacted with all the tested fish cell line antigens (Figs. 1 and 3). Best resolution of precipitin lines was usually observed in homologous reactions. Strong reactions were also observed when anti-EK-1 or anti-EO-2 serum rected with the tested antigens originated from eel kidney or ovary, respectively. However, no precipitin line was observed, when carp ovary and kidney antigens reacted with antiserum against EO-2 (Fig. 2).

Reciprocal immunoelectrophoresis test demonstrated that EK-1 and EO-2 cell lines were cross-reactive with the tested fish cell lines including EPC and RTG-2 (Figs. 4 and 5). Table 1 showed the number of precipitin lines resulted in immunoelectrophoresis plates. The results demonstrated that when anti-EK-1 serum reacted with EO-2 or EK-1 antigen respectively, the similar number and pattern of precipitin lines were observed. The identical results were also obtained when anti-EO-2 serum reacting with EK-1 or EO-2 antigen was performed. However, less precipitin lines were observed when anti-EK-1 or anti-EO-2 serum reacted with EPC or RTG-2 antigen, respectively. Immunoelectrophoresis plates of antigens originated from eel and common carp tissues relative to anti-EK-1 or anti-EO-2 serum were presented in Figs. 6 and 7. The results showed that various eel tissue antigens exhibited cross-reactive precipiting antigen



Figs. 1-3. Photomicrographs representing the image of fixed, stained immunodiffusion plates in reactions of anti-eel cell line (anti-EK-1 or anti-EO-2) sera with various antigenic complexes. aks: Anti-EK-1, aos: Anti-EO-2, eoc: EO-2 cell line antigen, ekc: EK-1 cell line antigen, epc: EPC cell line antigen, rtg: RTG-2 cell line antigen, eot: Eel ovary antigen, est: Eel spleen antigen, ekt: Eel kidney antigen, eht: Eel heart antigen, cot: common carp ovary antigen, ckt: common carp kidney antigen.



Figs. 4–7. Photomicrographs representing the image of fixed, stained immunoelectrophores plates in reactions of anti-eel cell line (anti-EK-1 or anti-EO-2) sera with various antigenic complexes. aks: Anti-EK-1, aos: Anti-EO-2, eoc: EO-2 cell line antigen, ekc: EK-1 cell line antigen, epc: EPC cell line antigen, rtg: RTG-2 cell line antigen, eot: Eel ovary antigen, est: Eel spleen antigen, ekt: Eel kidney antigen, eht: Eel heart antigen, cot: common carp ovary antigen, ckt: common carp kidney antigen.

(s) common to the tested eel cell lines (Table 1). No cross-reactive precipitin arcs were found when the antigens originated from carp reacted with antisera against EK-1 or EO-2 cell lines (Figs. 6 and 7; Table 1).

Discussion

Although immunoelectrophoresis is demonstrated to be useful in detecting similarities and differences amongst different molecules, EK-1 and EO-2 cell lines derived from tissues of Japanese eel (*Anguilla japonica*) were not differentiated using this approach. From the presence

Table I. Number of precipitin lines resolved in reactions between given antisera and antigens by using immunoelectrophoresis

Antisera	Antigens Derived from	No. of Precipitin Lines
Anti-EK-1	EK-1	5
	EO-2	5
	EPC	2
	RTG-2	1
	Eel Kidney	3
	Eel Ovary	2
	Eel Spleen	1
	Common Carp Kidney	0
Anti-EO-2	EK-1	3
	EO-2	3
	EPC	1
	RTG-2	1
	Eel Kidney	3
	Eel Ovary	4
	Eel Heart	2
	Common Carp Ovary	0

EK-1: Japanese eel (*Anguilla japonica*) kidney cell line EO-2: Japanese eel (*Anguilla japonica*) ovary cell line. EPC: Epithelioma papillosum cyprini tissue cell line; a cell line from *Cyprinus carpio*. RTG-2: Rainbow trout (*Salmo gairdneri*) gonad cell line.

of precipitin patterns, these two cell lines were distinguished from the other two cell lines which derived from the fishes belonging to families Cyprinidae and Salmonidae, suggesting that the serologic approach involving in the present study allowed distinction to be made at familic level. In the present study, the differentiation of fish cell lines was only performed by comparing the different degree of cross-reactivity of antigens. The perfect differentiation could be resulted from the availability of high specificity of antisera. The cross-adsorption of sera may provide a greater specific in the antisera, but, this is very time consumming and required a greater expenditure of effort. It is therefore, suggested that either immunodiffusion or immunoelectrophoresis is not ideal technique for the differentiation of fish cell lines with different origin. The presence of detective cross-reactive antigen between different cell lines may reflect the limitation in the application of immunodiffusion and immunoelectrophoresis on the characterization of fish cell lines.

The presence of precipitin lines between antisera against eel cell lines and eel tissues demonstrated the cell lines still possess their original property. In comparison, there were no common cross-reactive nature was obtained between eel cell lines and common carp tissues.

In the study of lepidopteran cell lines, Aldridge and Knudson (1980) demonstrated that differentiation of these cell lines at a familic level was possible by using serological techniques including complement fixation, hemagglutination, immunodiffusion and immunoelectrophoresis. They also suggested that immunoelectrophoresis was the best amongst the four used serological techniques for distinguishing lepidopteran cell lines. Similarly, the present study also demonstrated that the immunoelectrophoresis is better than immunodiffusion in the differentiation of

fish cell lines.

Apart from the serological techniques, the other approach, isozyme analyses, was reported to be superior to the serological techniques for the characterization of insect cell lines at an intrageneric level (Green, 1971; Greene *et al.*, 1972; Tabachnick and Knudson, 1980). In our laboratory, experiments are in progress which, it is hoped, will evaluate the application of isozyme analyses on the identification of fish cell lines.

中 文 摘 要

本研究乃利用雙向擴散及免疫電泳法，來評估此兩種方法應用於魚類細胞株之區別的可能性。實驗結果顯示，抗鰻魚卵巢及腎臟細胞株之免疫血清與其他魚類細胞株抗原有交叉反應作用。但由免疫電泳實驗所呈現之沈澱線數目，可判別同科或非同科之魚類細胞株。

又，利用雙向擴散及免疫電泳法亦證實了鰻魚細胞株與鰻魚組織抽取液間有共同抗原之存在。但若以鯉魚組織抗原與抗鰻魚細胞株血清作用等，則未有任何沈澱線出現。

Acknowledgments

We wish to thank financial support from Committee of Agricultural Planning and Development and National Science Council, Republic of China.

References

1. Aldridge, C. A. and D. L. Knudson (1980) Characterization of invertebrate cell lines. I. Serologic studies of selected lepidopteran lines. *In Vitro*, **16**(5), 384–391.
2. Chen, S. N. and G. H. Kou (1981) A cell line derived from Japanese eel (*Anguilla japonica*) ovary. *Fish Pathol.*, **16**(3), 129–137.
3. Chen, S. N., Y. Ueno and G. H. Kou (1982) A cell line derived from Japanese eel (*Anguilla japonica*) kidney. *Proc. Natl. Sci. Counc. B, ROC*, 6(1), 93–100.
4. Clarke, D. H. and J. Casals (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am. J. Trop. Med. Hyg.*, 7, 561–573.
5. Fogh, J. (1973) *Contamination in tissue culture.* New York, Academic Press, 1–288.
6. Greene, A. E. and J. Charney (1971) Characterization and identification of insect cell cultures. *Curr. Trop. Microbiol. Immunol.*, 55, 51–61.
7. Greene, A. E., J. Charney, W. W. Nichols and L. L. Corell (1972) Species identity of insect cell lines. *In Vitro*, 7, 313–322.
8. Lowry, O. H., N. J. Rosegrough, A. L. Farr and R. J. Randall (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
9. Tabanick, W. J. and D. L. Knudson (1980) Characterization of invertebrate cell lines. II. Isozyme analyses employing starch gel electrophoresis. *In Vitro*, **16**(4), 392–398.
10. Wolf, K. and J. A. Mann (1980) Poikilotherm vertebrate cell lines and viruses: A current listing for fishes. *In Vitro*, **16**(2), 168–179.