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Development and characterization of two new cell lines, CGL, and CGK from the liver and kidney of Channa Gachua

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Abstract
Two new cell lines (CGL and CGK) are developed from the liver and kidney tissues of *C. gachua*, (Ham), a freshwater wild fish severely affected with EUS. The tissues were disintegrated mechanically and seeded with 20% fetal bovine serum. The cells showed optimal growth at 25^0C with Egale's MEM's containing 5% L-glutamine. The major problem in the development of cell line from small size fish is slow growth rate *in vitro*, which is overcome by supplementing with the supernatant from the conditioned media from the culture of head kidney of the same species. The monolayer is obtained within 20 days of seeding and culture is observed up to 26 passages. The ultra-structural studies of these cell lines showed it's synthesizing and secretary in nature.

Keywords: Cell line, *C. gachua*, conditioned medium, MEM, CGL, CGK, SHRV.

1. Introduction
The cell culture is an essential tool for many studies of cellular metabolism, environmental toxicology and impact of drugs as well as virological studies. However, the major problem in cell culture is establishment and maintenance of small size individual due to their slow growth rate which ultimately slowdown the culture process. Although cultures from adult liver do not express all the properties of liver parenchyma, there is little doubt that the correct lineage of cells may be cultured. So far attempts to generating proliferating cell lines have not been particularly successful, but functional hepatocytes can be cultured under correct condition [1].
The kidney is a structurally complex organ in which the system of nephrons and collecting ducts is made up of numerous functionally and phenotypically distinct segments. This segmental heterogeneity is compounded by cellular diversity that has yet to be fully characterized. Some tubular segments possess several morphologically distinct cell types. In addition, evidence points to rapid adaptive changes in cell ultra-structure that may correlate with changes in cell function [2]. This structural and cellular heterogeneity presents a challenge to the cell culturist interested in isolating pure or highly enriched cell populations. The difficulty of the problem is further compounded for studies of the human kidney, where form and access to the specimen may make some manipulations such as vascular perfusion difficult or impossible. In case fishes, this condition is more crucial in case of fishes because it consists of one more tissues *i.e.* lymphoid tissues, which makes the culture more complicated.
Several approaches have been used successfully to culture the cells of specific tubular segments. Density gradient methods are now commonly used to isolate enriched populations of enzyme-digested tubule segments, and are particularly effective in establishing proximal tubule cell cultures from experimental animals [3]. Specific nephron or collecting duct segments can also be isolated by microdissection, and then explanted to the culture substrate. Immunodissection [4] and immunomagnetic separation [5] methods have also been developed to isolate specific nephron cell types on the basis of expression of cell type-specific ecto-antigens. These elegant methods hold considerable promise for the study of specific kidney cell types in health and disease, but as yet have been applied almost exclusively to studies on experimental animals. The limited (and unscheduled) availability and inconsistent form (*e.g.*, excised pieces, damaged vasculature, lengthy postnephrectomy period) of human donor kidneys make progressive enzymatic dissociation a more practical means to isolate human kidney cells for culture.

2. Material and Methods

2.1. Development of Fish Cell Lines: For the propagation of virus, two cell lines, CGL/14 and CGK/14 were developed from the liver and kidney of *Channa gachua*, respectively as per procedure given by Chen *et al.* [6]. For the present work of cell culture, certain modifications were added. Briefly the process could be discussed as follows. The kidney and liver were taken out aseptically and the cells were disintegrated mechanically with the help of mortar and pestle, which was then centrifuged at 300x g for 15 min. To observe the impact of addition of L-glutamine on growth of culture, two different sets of culture flasks were kept with and without addition of 5% L-glutamine. Similarly to observe the effect of temperature, five flasks are kept at five different temperature ranged between 20-30^{0}C at interval of 2^0 C.

2.2. Growth of Cell lines, Infection and observations of Cellular details: In all the cases, growth of culture are observed by counting of cell number was done traditional neubaure chamber method. For the experimental purpose and to achieve the better result, addition of 2 ml supernatant of conditioned media from the culture of head kidney of the same species is done in all flasks except one left without it and designated as standard. The identification of cell is done morphologically, with the help of ultra-structural study as per procedure given by Madeley [7]. Both the lines are tested for susceptibility against the SHRV (RV-19E) as per procedure given by Plumb [8].

3. Observations

3.1 Primary Culture: Flasks seeded with the liver cell of *Channa gachua* were observed daily under inverted tissue culture microscope after the primary culture. No multiplication of cells is observed in standard flask while optimal growth is observed in the flasks containing conditioned media. The epithelial and fibroblastic cells are observed migrating from tissue fragments from every flask. After 3-4 days, several colonies consisting of epithelial cells were observed in various parts of flasks. These epithelial cells seen in 12 flasks multiplied to form colonies and terminated the growth in manner of contact-inhabited monolayer in 27-38 days these colonies were calloginized and reseeded in a new flasks. Cells growths were not observed in eight flasks and fibroblastic cells grow predominant in other flasks. The maximum growth is observed at 25^{0}C and similarly the flask containing L-glutamine showed comparatively better growth.

3.2. Passage of CGL: The secondarily passaged five cells, CGL-7, -8, -15 and –17, showed an epithelial morphology and grew to form a cell sheet until the contact inhibition restricted the growth. From the fifth passage of CGL-8, 12 and 14 shortened its doubling time gradually, and were able to transferred at the interval of seven to ten days after seventh passage when seeded at the density of 1,00,000 cells/ flasks. CGL-14 has been subculture for 90 times during 18 months (Fig.1).

3.3. Morphology of CGL-14: CGL-14 was consisted of rounded nucleus and abundant granular intracytoplasmic organelles. A few fibroblastic spindle cells with the small nucleus were observed CGL-14 have no PAS positive granules even culture in 199 –10 medium supplemented with 0.1% glucose.

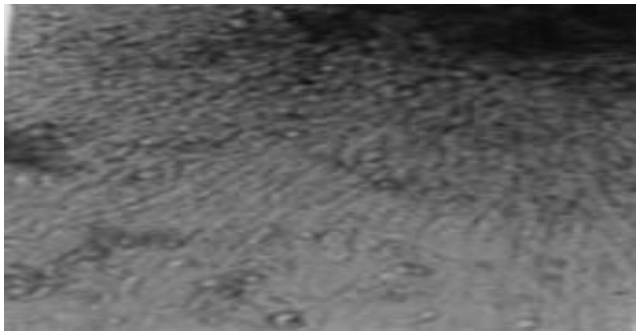

Fig 1: Culture of liver cells (x450)

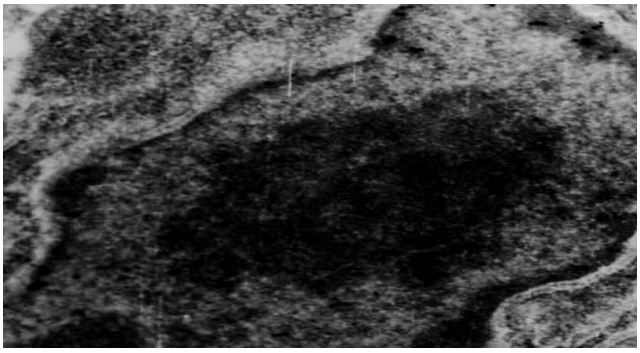

Fig 2: single liver cell (x55.5k)

3.4. Passage of CGK: The secondarily passaged five cells, CGK, -8, 10, 14 and 18, showed an epithelial morphology and grew to form a cell sheet until the contact inhibition restricted the growth. From the fifth passage of CGK-8, 10 and 14 shortened its doubling time gradually, and were able to transferred at the interval of seven to ten days after seventh passage when seeded at the density of 1,00,000 cells/ flasks. CGK-14 has been subculture for 90 times from during 18 months.

3.5. Morphology of CGK: CGK-14 was consisted of rounded nucleus and abundant granular intracytoplasmic organelles. A few fibroblastic spindle cells with the small nucleus were observed CGK-14 have no PAS positive granules even culture in 199–10 medium supplemented with 0.1% glucose (Fig. 2).

3.6. Susceptibility to Cell Lines: The infectivity of SHRV was compared by using plaque-forming assay on tow cell lines CGL-14 and CGK-14 (Tabs.1-2). Both of the cell lines were found to be susceptible to SHRV but CGL-14 was slightly higher susceptible than CGK-14 (Figs.3-4).

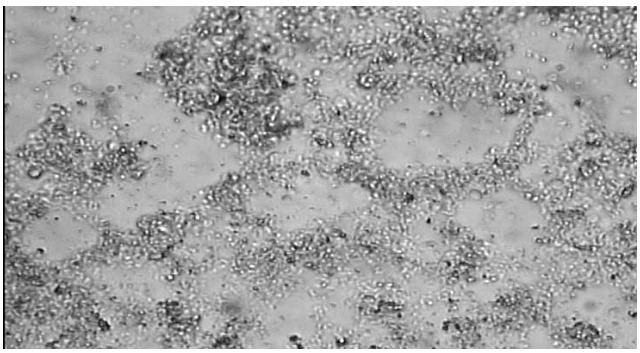

Fig 3: Culture of liver cell culture after inoculation of SHRV

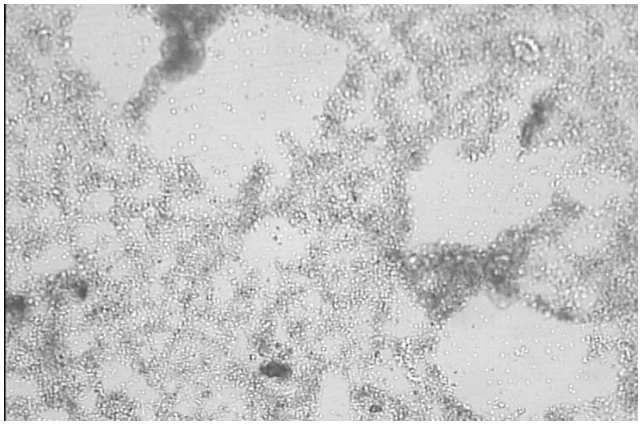

Fig 4: Culture of kidney cell culture after inoculation of SHRV

3.7. Electron Microscopy (TEM): Under electron microscope, two different types of cells are observed *i.e.* hepatocytes, and renal cells. In a cell of kidney the cellular structure is found as usual and all the organelles are observed. The mitochondria and channels of ER were intact. Normal cellular architecture could be noticed in liver cell. Most of the cells were large and round with a round nucleus. The cells had smooth surface. In this case the ER, mitochondria, Golgi bodies were found to be intact, rather indicating the high rate of protein synthesis as indicated by the comparatively high number of ER and size of nucleus were also found to be increased (Fig. 5-6).

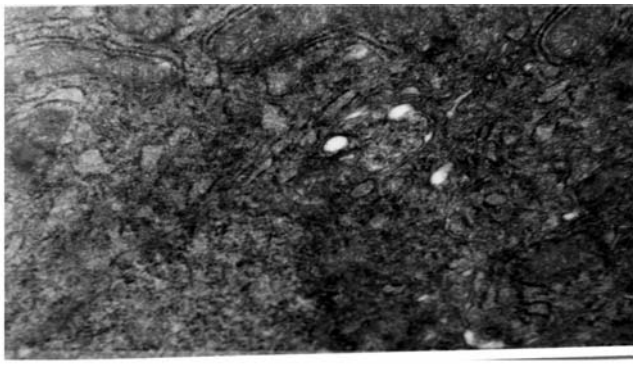

Fig 5: A section of a liver cell under electron microscope (x65.5k)

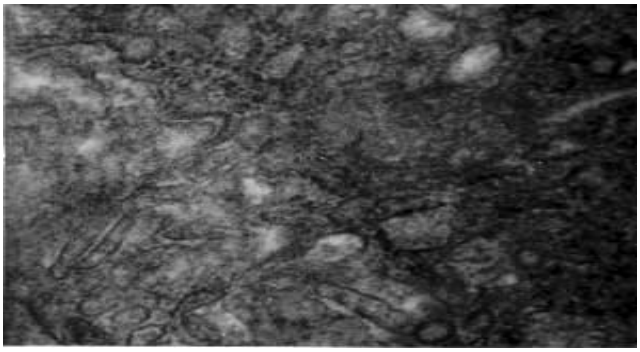

Fig 6: A section of a renal cell under electron microscope (x65.5k)

Table 1: Effect of Rhabdovirus (RV-19E) on the cell line, CGK/14

Viral dilution	Mortality ratio	Died	survived	Total dead	Total survived	Cumulative ratio	Death %
10^{-1}	6/6	6	0	19	0	19/19	100
10^{-2}	6/6	6	0	13	0	13/13	100
10^{-3}	5/6	5	1	7	1	7/8	88
10^{-4}	2/6	2	4	2	5	2/7	29
10^{-5}	0/6	0	6	0	11	0/11	0

Table 11: Effect of Rhabdovirus (RV-19E) on the cell line, CGL/14

Viral dilution	Mortality ratio	Died	Survived	Total dead	Total survived	Cumulative ratio	Death %
10^{-1}	6/6	6	0	16	0	16/16	100
10^{-2}	5/6	5	1	10	1	10/11	91
10^{-3}	4/6	4	2	5	3	5/8	63
10^{-4}	1/6	1	5	1	8	1/9	11
10^{-5}	0/6	0	6	0	14	0/14	0

4. Discussion

The liver and kidney cells transferred from fingerling to culture flask multiplied with comparatively at rapid rate and took 28-36 days for primary culture. The flask containing L-glutamine showed comparatively more growth. L-glutamine was added as essential amino acids in the culture medium. The secondarily passaged five cells, CGL-7, -8, -15 and –17, showed an epithelial morphology and grew to form a cell sheet until the contact inhibition restricted the growth. The secondarily passage five cells, CGK, -8, 10, 14 and 18, showed an epithelial morphology and grew to form a cell sheet until the contact inhibition restricted the growth. From the fifth passage of CGK-8, 10 and 14 shortened its doubling time gradually, and were able to transferred at the interval of seven to ten days after seventh passage when seeded at the density of 1,00,000 cells/ flasks. CGK-14 has been subculture for 90 times during 18 months. Most of the tests by using mammalian heapatocytes [9, 10], have been performed to culture liver cells with differentiated hepatic function. Similarly, the tests used in mammalian kidney cells [11] have been performed to culture these cells with differentiated renal function.

Both of the cell lines were found to be susceptible to SHRV but CGL-14 was slightly higher susceptible than CGK-14 which is in accordance with the report of many workers including Yoshimizu, *et.al.* [12], which showed the susceptibility of fish cell lines with salmonid viruses. The infectivity of SHRV was compared by using plaque-forming assay on tow cell lines CGL-14 and CGK-14. These cell lines when inoculated with different dilutions of virus, the virus titter was recorded to be $10^{3.34}$ $TCID_{50}$/volume inoculated. EUS-associated rhabdovirus isolated from these cell lines, resembles with BPV structurally. The virus isolated is nominated as RV-19 E. The dimensions noted for RV-19E under this investigation tally with those reported by Frerichs *et al*. [13] and Kasornchandra *et al.* [14].

5. References

1. Guguen-Guillouzo C. Isolation and culture of animal and human hepatocytes: in Freshney R.I. edn: Culture of Epithelial Cells. New York Wiley Liss, 1992, 198-223.
2. Stanton BA, Viemesderfer D, Wade JB, Giebisch G. Structural and functional study of the rat distal nephron:Effects of potassium adaptation and depletion.*Kidney Int* 1981; 19:36-48.
3. Taub ML, Yang SI, Wang Y. Primary rabbit proximal tubule cell culture maintain differentiated functions when cultured in a hormonally define serum-free medium. *In vitro cell Dev. Biol* 1989; 25:770-775.
4. Smith, Garcia-Perez A. Immunodissection: Use of monoclonal antibodies to isolate specific types of renal cells: *Am J.Physiol* 1985; 248:F1-F7
5. Mathews REF. Classification and nomenclature of viruses. Third Report of the International Committee on Taxonomy of Viruses. Intervirology Nos 1979; 12:3-5.
6. Chen SN, Ueno Y, Wen SC, Kou GH. Establishment of a cell line from kidney of *Tilapia*, Bull, Eur. Ass. Fish Pathol 2003; 3:1-4.
7. Madeley CR. Traditional techniques of viral diagnosis: Medical Virology (A Practical Approch) (Edited by Desselberger. 1995), Oxford University press, ledn, 1995, 1-32.
8. Plumb JA. Microbial fish disease laboratory manual, Brown printing company Montgomery, Alabama, 1983, 1-95.
9. Richman RA, Claus TH, Pilkas LJ, Friedman DL. *Proc Natl. Acad. Sci.* USA 1976; 73:3589-3593.
10. Guguen-Guillouzo C, Clément B, Baffet G, Beaumont C, Morel-Chany E, Glaise D *et al.*, Maintenance and reversibility of active albumin secretion by adult rat hepatocytes co-cultured with another liver epithelial cell type. Exp Cell Res 1983; 143(1):47-54.
11. McAteer JA, Kampson SA, Evan AP. Culture of Human Cortex Epithelial Cells. *J. Tissue Cult. Methods* 1991; 13:143-148.
12. Yoshimizu M, Kamel M, Dirakbusarakom S, Kimura T. Fish cell lines Susceptibility to salmonid viruses.In Kuroda, Y.; Kurstak, E. and Maramorosch, K. (Eds). Invertebrate and fish tissue culture, Japan. Sci. Soc. Press. Tokyo, 1987, 218.
13. Frerichs GN, Millar SD, Roberts RJ. Ulcerative rhabdovirus in fish in Hedrick, R.P., Eaton, W.D., Fryer, J.L., Groberg, W.G. Jr, Boonyaratpalin, S. (1986) Characteristics of a birnavirus isolated from cultured sand goby (*Oxyeleolris marmoratus*). *Dis. Aquat. Org* 1986; 1:219-225.
14. Kasornchandra J, Engelking NM, Lannan CN, Rohovec JS, Fryer JL. Characteristics of the rhabdoviruses from snakehead fish *Ophicephalus striatus*. Dis. Aquat. Organisms 1992b; 13:89-94.