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Original Article

Evaluating of Iran Razi Khedmati Bovine Kidney (IRKHBK) Cell Line for Isolation and Propagation of Viruses

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Abstract

Background and Aims: To identify and isolation of viruses a susceptible cell is needed, which can be primary or line. The use of primary cells is laborious, time consuming and expensive, while the use of cell lines is much easier and faster. A cell line was originated from primary cells of bovine kidney by continuous passage. The aim of this study was to introduce this cell line for isolation, propagation and diagnosis of animal viruses.

Materials and Methods: Samples of the infected animals, such as the lymph nodes, tonsils, lungs and blood were used for virus isolation. Primary BK cell and IRKHBK cell line cultures were inoculated with the prepared samples simultaneously under the same conditions. Identification of the isolated viruses was carried out with a virus neutralization test using standard antibodies.

Results: Bovine Herpes Virus-1, Parainfluenza-3, Bovine viral diarrhea, and Peste des Petits Ruminants Virus were easily isolated and propagated in this cell line. The viral titer was higher in theses cells as compared to the primary BK cell. This cell line designated as IRKHBK (Iran Razi Khedmati Bovine kidney).

Conclusion: The results show that this cell line can be used for virus isolation, detection and propagation. For both animal and human viruses for research and diagnostic tests.

Keywords: IRKHBK; Cell line; Virus Isolation; Primary Cell Culture

Introduction

Since 1948 that Weller and Enders have described the use of cell culture for virus isolation, the technique has become a main tool for isolation of viruses from clinical samples (1). Despite the development of new methods to identify pathogens, cell culture is still of great importance for providing an opportunity to investigate the biological properties of isolated viruses (1-4). Unlike most of the antigen and nucleic acid detection methods, cell culture allows simultaneous detection of multiple viruses in the sample (1). Animal cells are widely used even for the isolation and propagation of pathogenic viruses in diagnostic laboratory. Viral growth in cells leads to the appearance of morphological changes and occurrence of cytopathic effect (CPE) (1, 2). The different characteristics of CPE are related to the type of viruses. The cells may become round and gradually separate from the context, or merged and create large cells with multiple nuclei as giant cells.

Several types of cells are used for isolation of different viruses in the diagnosis virology laboratories (1, 2). The cell culture system has also led to propagating, counting and titration of the viruses. Moreover, studying of the new emerged virus strains and their characteristics

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will be easier. Cell culture can be classified into three groups: primary cell cultures as monolayer, diploid cell cultures or semi-continuous, and continuous or immortal cell lines. The organs of live animals or embryonic cells can be used to produce primary cell culture. Preparation of the cells is laborious, time consuming and expensive due to their finite lifespan (5). Such problems can be solved by using cell line (5, 6). Manipulating of cell line is much easier and less costly than primary cell. The established cells must pass all stages of quality control tests and be free from microorganisms that may be present in live animals (7). A variety of cell lines from different organs are genetically developed by inserting the T antigen of the simian virus 40 (SV40), the E6E7 gene of human papilloma virus or a telomerase gene into genome of primary cells (2, 8-10). The cytogenetic feature of the cell has been documented previously (11). This cell line can be used in diagnosis of viral infectious diseases, isolation and propagation of viruses. In this study, we have successfully developed and introduced a cell line designated as IRKHBK (Iran Razi Khedmati Bovin Kidney).

Methods

IRKHBK cell line

Cells were prepared in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum and antibiotics (12). The cells were incubated at 37 ° C until 80-90% confluency was formed. Viability of the collected cells was estimated by using trypan blue at a concentration of 0.4%. Then 1-2×105/cm 2 cells/ml were transferred to another flasks and 10 ml complete medium was added. The flasks were incubated at 37°C to reach to the appropriate cell density for the next passage. Flasks were observed daily to ensure uniform growth of cells. The cells were ready for sample inoculation three days after culture.

BK primary cells

Kidney of calf was cut with sterile scissors into small pieces. The tissue fragments were washed three to four times with phosphate buffer saline (PBS) and digested by 0.25% trypsin solution for one hour at 37° C. Then a few drops of fetal bovine serum were added to neutralize the trypsin. The digested organ was mixed with buffer containing antibiotics and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed under sterile conditions and the pellet was diluted to a ratio of 1 to 500 with MEM medium. A concentration of 5×105 - 1× 106 cells / ml was transfer to other flasks containing complete MEM medium and incubated at 37 ° C.

Viruses and Antiserum:

Parainfluenza type 3 virus (PI3), bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV1) and Peste des Petits ruminants (PPR) and their antiserum were used for virus neutralization test.

Preparation of specimens

Tissue samples including lymph nodes, tonsils, testis, lungs and eyes of dead or affected animals, and blood samples of animals suspected to parainfluenza infection were collected. The tissues were processed by cutting with scissor and forceps and grounded in sterile PBS to make a 20% suspension of tissue in PBS containing antibiotic. The suspension was centrifuge at 2000 rpm for 10 min and the supernatant was stored in -20 ° C until used.

Inoculation of the suspected specimens

All operations were performed in sterile conditions according the OIE (6). The culture media of confluent IRKHBK cell line were placed in a sterile container before inoculation of the specimens. Two ml of the suspension of 10% of the homogenized tissues were inoculated in the IRKHBK cells without media and the cells incubated at 37 °C for 30 minutes. Then about 1.5 ml MEM medium containing 2% serum and antibiotics were added to each flask. One uninfected-cell flask was used as control. Simultaneously the same procedure was used for inoculation of the tissue specimens into the BK cells. The cultures were controlled daily with inverted microscope. In the absence of CPE, culture suspensions were frozen and thawed for three to four subsequent passages.

Hemadsorption test

The medium of cultures was discarded and cells were washed with PBS. 0.1% suspension of red blood cells of guinea pigs was added to the culture tube. The tubes were incubated for 30 minutes at 37°C after which red blood cells were discarded. After washing cells with buffer they were examined with inverted microscope.

Virus neutralization test (VNT):

The VNT was performed according to the OIE procedure (6). Briefly the standard serum was inactivated at 56°C and then ½ dilution of antiserum was mix with the equal volume of infected cell suspension (V:V). The mixtures were incubated for one hour at 37°C then each sample was inoculated into five IRKHBK and five BK cells culture. Control of virus, negative and positive sera were also examined. Cell cultures were observed for 7 days and the results were recorded.

Virus titration: The serial 10-fold dilutions of both infected cells were prepared and each dilution was inoculated into 5 tube of IRKHBK or BK cell culture. After 7 days incubation at 37°C, the titer of virus was calculated by Reed and Muench method (13).

Staining the cells

The media of cultures was removed and the cells were washed once with PBS. The cells were fixed on the cover slip with 4% formalin for one hour then placed in crystal violet 5% (W/V) for 5 minutes. The cultures were washed and dried at room temprature.

Results

All of the examined viruses were isolated is a shorter time in IRKHBK cell line than primary BK cells (Table 1). Depending on the type of virus and cell susceptibility, the CPE appeared gradually one to six days after inoculation of tissue samples into the cell culture. The results showed that the IRKHBK cell line is more susceptible for virus infection and the marked CPEs were appeared and faster than the primary BK cell. The un-infected, BHV1 and BVD IRKHBK-infected cells are shown in figure 1.

The hemadsorption was used for detection of viruses that produced either slight CPE in cell culture. Thus the presence of PI3 virus in cell culture was confirmed by hemadsorption according to Elizabeth *et al* (14). Identification of the isolated viruses in IRKHBK and BK cells was carried out by VNT which revealed the great susceptibility of IRKHBK cell line for the viruses' replication. Results of virus titration in both IRKHBK cell line and primary BK cell are shown in table 2. The virus titers were calculated to be at least one logs higher in IRKHBK cell line culture than primary BK cell.

Discussion

Early diagnosis of most viral diseases is possible based on the clinical symptoms but definitive diagnosis requires laboratory findings based on virus isolation. Primary isolation and detection of viruses in cell culture is sensitive and has highest specificity. Although cell culture is a time consuming assay for virus detection, it still has an important role in the antigenic and genetic

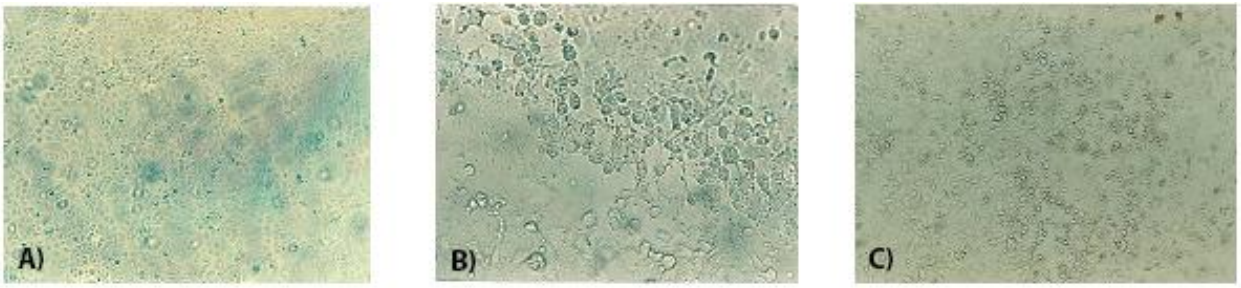

Fig. 1. Uninfected IRKHBK cells used as control (A). Cytopathic effect induced in IRKHBK cells by BHV-1(IBRV) 2 days post inoculation (B) and BVDV 4 days post inoculation (C). (40X magnification).

Table 1: Susceptibility of IRKHBK cell to viruses' infections based on CPE observation duration.

Virus / Cell type	BHV-1	PI3	RPV	BVDV	PPRV
IRKHBK	**1-2***	**2-3**	**3-4**	**4-5**	**5-6**
BK	**3-4**	**4-5**	**5-6**	**6-7**	**7-8**

* Days post virus inoculation

Table 2: Adapted virus titers log 10 ($TCID_{50}$) estimated in IRKHBK cell cultures

Virus / Cell type	BHV-1	PI3	RPV	BVDV	PPRV
IRKHBK	**6.0 – 6.5**	**4.5 - 5**	**5.5 – 6.0**	**3.5 – 4.0**	**4.5 – 5.0**
BK	**5.0**	**3.5 – 4.0**	**4.5 – 5.0**	**3.0 – 3.5**	**3.5 – 4.0**

studies of viruses.

A variety of cell lines have been produced using genetic methods for proper replication and propagation of viruses (7-10, 15). The development and the use of living organisms that can be manipulated by using genetic and recombinant techniques have been used and controled in recent years. Therefore, an originated-animal cell line that is established without using genetic techniques is essential in virology research and for diagnosis of infectious diseases in domestic animals. IRKHBK cell lines originated from primary bovine kidney cells was obtained by successive passages (11). In this study, the susceptibility of this cell line for proliferation and isolation of animal viruses was investigated. Preliminary results indicated that after inoculation of tissue specimens of affected animals into IRKHBK cell line, viruses caused CPE faster than in BK cell. Confirmation of isolated viruses was performed by virus neutralization test. Virus titers were measured after two or three passages of the viruses in the cell line. As shown in table 1 viral titers of isolated viruses in IRKHBK cell line were higher than those in BK cell grown.

Cell culture is used as a tool for isolation, diagnosis and characterization of viruses. A variety of cells originated from animal tissues have been widely used in virology (23). The bovine kidney and lung primary cells are most useful cells for isolation and propagation of bovine viruses. But in some circumstances preparation of fresh primary cells that was free from adventitious virus is difficult, especially in cases where there may be factors such as bovine spongiform encephalopathy (7). In such cases, it is difficult to be sure that animal is free from viral diseases. In this research, susceptibility of IRKHBK cell line for detection, propagation and isolation of bovine viruses in comparison with primary BK cells was reported. In parallel to this study, ability of IRKHBK cell line for isolation of humans and animals viruses was investigated (16 - 20, 21). In addition to viruses, the susceptibility of this cell line for the propagation of other microorganisms has been investigated. Cultivation of Pneumocystis Carinii, a common infection in immune compromised patients, in artificial chemical environments was not successful. The possible growth of the parasite was examined in IRKHBK, Vero and MRC5 cells. The result indicated a significant difference (P= 0.023) between IRKHBK and other cells five days after inoculation. This means that IRKHBK cell is also suitable for propagation of Pneumocystis carinii (22).

Taken together the results of this study confirmed that the IRKHBK cell is more susceptible cells for isolation and growth of viruses.

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