RAPID COMMUNICATION

Is parainfluenza virus a threatening virus for human cancer cell lines?

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Abstract

Immortalized cell lines, such as human cancer cell lines, are an indispensable experimental resource for many types of biological and medical research. However, unless the cell line has been authenticated prior to use, interpretation of experimental results may be problematic. The potential problems this may cause are illustrated by studies in which authentication of cell lines has not been carried out. For example, immortalized cell lines may unknowingly be infected with viruses that alter their characteristics. In fact, parainfluenza virus type 5 (PIV5) poses a threat to the use of immortalized cell lines in biological and medical research; PIV5 infection significantly alters cellular physiology associated with the response to interferon. If PIV5 infection is widespread in immortalized cell lines, then a very large number of published studies might have to be re-evaluated. Fortunately, analyses of a large number of immortalized cell lines indicate that PIV5 infection is not widespread.

Key words: authentication, cell bank, cell line, parainfluenza virus, quality check.

INTRODUCTION

Immortalized cell lines, such as human cancer cell lines, are very useful and indispensable experimental materials in current biology and medical science. However, without the authentication of such cell materials prior to use, experimental results using cell materials don't make sense.

Parainfluenza virus type 5 (PIV5), a paramyxovirus that harbors a non-segmented negative-stranded RNA genome, was originally isolated from simian kidney cell cultures and was called simian virus 5 (SV5). Subsequently, the virus has also been isolated from various mammalian species such as dogs, pigs, and humans. In dogs, PIV5 is responsible for a respiratory disease.[1]

The following report by Young *et al.* was surprising: "AGS and other tissue culture cells can unknowingly be persistently infected with PIV5; a virus that blocks interferon signaling by degrading STAT1".[2] When screening various cell lines for their ability to respond to interferon (IFN), the authors noted that in comparison to other tissue culture cells AGS tumor cells, which are widely used in biomedical research, had very low levels of STAT1. The reason for this was that AGS cells were persistently infected with PIV5, a virus that blocks the IFN response by targeting STAT1 for proteasome-mediated degradation.

Thus, the AGS cell line infected with PIV5 is not appropriate material to analyze the mechanism by which gastric cancer acquires resistance to IFN. The authors cautioned that some of the conclusions drawn from previous studies that used AGS cells may have to be reevaluated, especially as not only does the V protein of PIV5 target STAT1 for degradation, but it also binds to, and inhibits the activity of, MDA-5, a cellular protein that recognizes dsRNA and activates the IFN induction cascade.[3,4]

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In addition, PIV5 appears to infect a wide variety of cells including an oligodendrocyte cell line.[2] Thus, we evaluated the possibility of PIV5 infection for the human cancer cell lines deposited in our cell bank so as to exclude the erroneous interpretation due to the use of cells infected by PIV5.

METHODS

Cells

The AGS cell line was obtained from the American Type Culture Collection (ATCC, http://www.atcc.org/; Monassas, VA, USA). Other cell lines were obtained from the Cell Engineering Division of RIKEN Bio-Resource Center (RIKEN BRC, http://www.brc.riken.jp/lab/cell/english/; Tsukuba, Ibaraki, Japan). The human cancer cell lines analyzed in the present study are listed in Table 1.

Western blot analysis

Total cell extracts were generated by lysing cells (1 × 10^7) in CellLytic-M (Sigma-Aldrich, St. Louis, MO, USA). After removing cell debris, protein concentration was quantified with Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Ten micrograms of protein samples were electrophoresed through sodium dodecyl
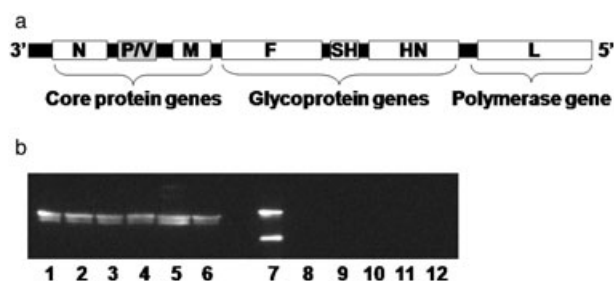
Figure 1 Genomic structure of parainfluenza virus type 5 (PIV5) and an example of a screen for PIV5 infection. (A) Genomic structure of PIV5. PIV5 possesses eight genes and produces nine different proteins. P and V proteins are produced from the same coding region by RNA editing. Thus, P and V proteins commonly possess the same N-terminal amino acid sequences. (B) An example of a western blot analysis. β-actin was used as the internal control for the amount of protein (lanes 1–6). A monoclonal antibody against both P and V proteins was used to detect PIV5 infection (lanes 7–12). The AGS cell line was used as the positive control. Lanes 1 and 7, protein sample prepared from AGS cell line; lanes 2 and 8, Lu-138 cell line; lanes 3 and 9, HLC-1 cell line; lanes 4 and 10, MIA Paca2 cell line; lanes 5 and 11, S1 cell line; lanes 6 and 12, MR10-1 cell line. The protein sample prepared from AGS cells clearly showed two bands for both P and V proteins (lane 7), whereas the other cells did not.

Table 1 List of cell lines used for screening

Screening	Cell lines
Esophageal cancer	TE-1, TE-4, TE-6, TE-8, TE-10, TE-11, TE-14, TE-15, EC-GI-10
Gastric cancer	HGC-27, NUGC-4, MKN1, MKN7, MKN45, MKN74, Kato III, HuG1-PI, HuG1-N, GCIY, SF8657, ECC10, ECC12, LMSU, TGBC11TKB, KE-39, KE-97, H-111-TC, SH-10-TC, AZ521, NCC-StC-K140, GSS, GSU
Colon cancer	CW-2, CACO-2, COLO-320, LoVo, JHCA-ov, COLO205, TT1TKB, PMF-ko 14, JHCOLOYI, JHSK-rec
Hepatic cancer	HuH-7, Hep G2, Li-7
Bile duct cancer	TGBC24TKB, HuH-28, HuCCT1, RBE, SSP-25, TKKK
Pancreatic cancer	PK-1, PK-45H, PK-45P, PK-59, MIA Paca2, PANC-1, NOR-P1
Breast cancer	ZR-75-1
Oral cavity cancer	T3M-1 Cl-10, CJM, HSQ-89, Sa3, T3M-1 Clone2, HSC-2, HSC-3, Ho-1-u-1
Cervical cancer	OMC-1, TCS, HeLa, BU25TK-, TC-YIK, HOKUG, SKG-II-SF, QG-U, TCO-2, TOM-2, JHUS-nk1, JHUCS-3
Sub-lines of HeLa cell line:	MR10-1, HEp-2, HeLa TG, HeLa.S3, HeLa.S3(SC), MR1-3, MR6, HeLa.S3(Mer∧(-)), HeLa P3
Urinary bladder cancer	5637
Lung cancer	RERF-LC-AI, RERF-LC-KJ, Sq-1, EBC-1, LK-2, LC-1/sq-SF, LC-1F, LC-1/sq, MS-1, Lu-134-A, Lu-134-B, Lu135, Lu-138, Lu-139, Lu-140, Lu-141, Lu-143, Lu-165, T3M-12, S1, 87-5, WA-hT, S2, IA-LM, 86-2, IA-5, A549, II-18, LU65, 633, Lu-24, HLC-1, LC-2/ad, PC-14
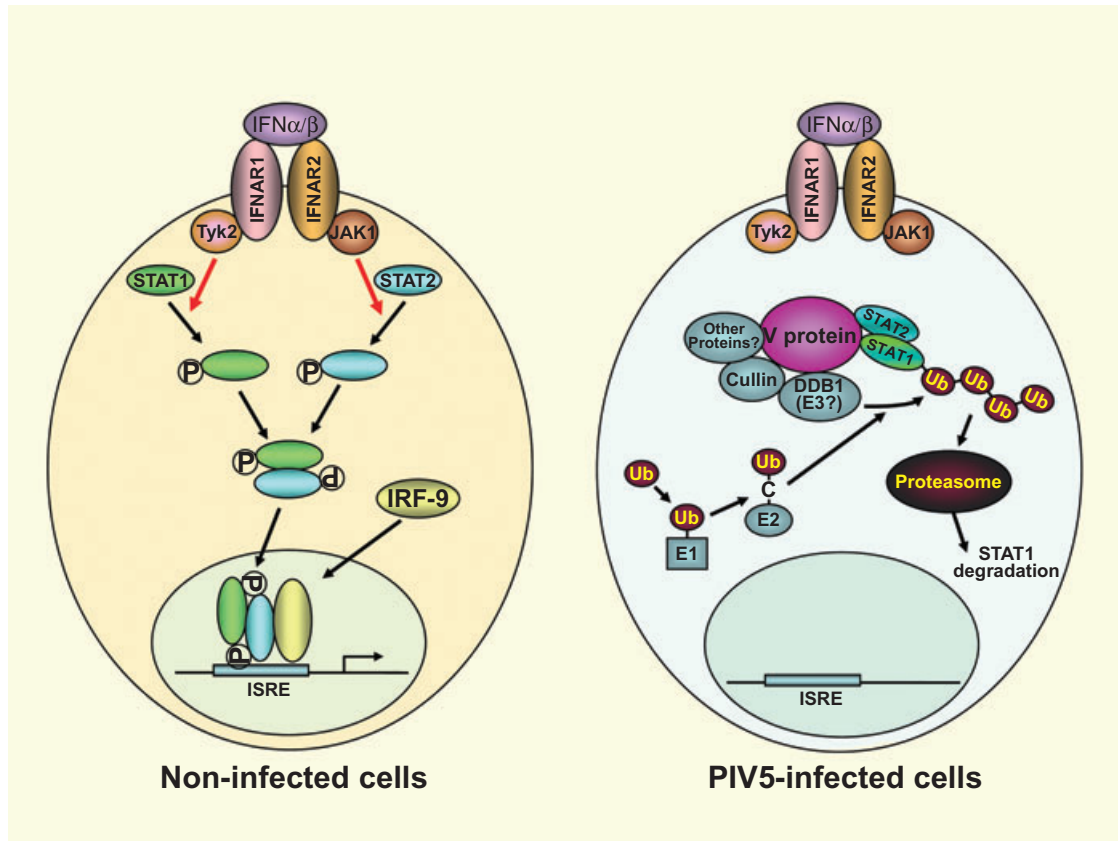
Figure 2 Mechanism of STAT1 degradation by the V protein of parainfluenza virus type 5 (PIV5).

sulfate (SDS)-15% polyacrylamide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were incubated with either a monoclonal antibody (MoAb) against P/V protein produced by PIV5 (AbD Serotec, Oxford, UK) or a MoAb against β-actin (an internal control; Abcam, Cambridge, UK), and bound antibody was revealed by horseradish peroxidase-conjugated goat anti-mouse IgG (GE Healthcare, Fairfield, CT, USA) by using enhanced chemiluminescence (Pierce, Rockford, IL, USA).

RESULTS AND DISCUSSION

Parainfluenza virus type 5 can infect cells derived from the stomach, such as AGS cells and oligodendrocytes.[2] In addition, respiratory tract epithelial cells and bone marrow cells can be infected by PIV5.[1] At the moment, we cannot rule out the possibility of PIV5 infection of any cell type. Thus, we evaluated the PIV5 infection status of many cancer cell lines derived from various tissues (Table 1).

The genomic structure of PIV5 is shown in Figure 1A. To detect PIV5 infection, we carried out western blot analyses, using a monoclonal antibody against the P/V proteins of PIV5, on cell lysates prepared from each cell line. We used the AGS cell line as a positive control in these analyses; Young *et al*. reported previously that this cell is infected with PIV5.[2] We analyzed 123 of the approximately 600 human cell lines deposited in our cell bank (see Methods, Table 1). Fortunately, PIV5 infection was not detected in any of the cell lines analyzed in this study. One example of a western blot analysis is shown in Figure 1B; the results from the other such analyses are not shown.

Of note, the authentication of immortalized cell lines has not necessarily been accomplished. For example, misidentification of cell lines has frequently been detected.[5–8] BBC radio also recently reported this fact with a 40-min broadcast titled "Cancer studies wasted millions" (20 November 2007). Short tandem repeat (STR) polymorphism analysis to exclude misidentification among human cell lines is a very powerful method[9,10] and is routinely carried out at present in cell

banks around the world. Thus, if researchers would use only the cell lines that have been tested by STR polymorphism analysis in cell banks, misidentification of human cell lines will be eradicated in the future.

On the other hand, virus infection is another critical issue in the use of immortalized cell lines. First, biohazard of cell lines should be excluded. Thus, in relation to the cell lines derived from liver cells, we routinely evaluate the infection of hepatitis B and C viruses (HBV and HCV). In relation to the cell lines derived from hematopoietic cells, we routinely evaluate the infection of human immunodeficiency virus type 1 (HIV1) and human T cell leukemia virus type 1 (HTLV-1). Second, the effects of virus infection on the characteristics of the infected cell lines must be taken into account, since it is clear that alteration of cell characteristics by virus infection leads to erroneous interpretation of the experimental results.

The AGS cell line infected with PIV5 is a sub-clone that has been deposited in a cell bank; the parental AGS cell line was not infected with PIV5.[2] Thus, the PIV5 infection of the AGS sub-line appears to have occurred during the culture prior to the deposition of the sub-line in the cell bank. Since PIV5 can infect the cells of various animal species,[1] PIV5 may be present in some of the materials typically used for cell culture, such as serum. Although the infection of AGS cells appears to be a secondary event during culture, it is still possible that primary cancer cells may be infected with PIV5 and that this infection produces resistance to IFN in the clinic.

Although the cell lines deposited in our cell bank and tested in this study were free of PIV5 infection, we want to emphasize the importance of testing PIV5 infection for immortalized cell lines, especially when they are used to analyze the mechanism of signal transduction of IFN (Fig. 2). In addition, it is important for the scientific community to be aware that cells may be persistently infected with viruses that significantly alter cellular physiology. Since many viruses able to infect cell materials are present and the effects of such infection on cellular physiology are largely unknown, the authentication of immortalized cell lines regarding virus infection is necessary and should be continued enthusiastically in cell banks around the world as one of the most pivotal missions.

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