BRIEF COMMUNICATION

Presence of Mason-Pfizer Monkey Virus in Some Stocks of the Human HBL-100 Mammary Epithelial Cell Line

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The HBL-100 cell line, established from primary cultures of milk epithelial cells of a healthy woman (1,2), owes its transformed phenotype to integrated simian virus 40 (SV40) genetic information (3). The cell line lacked malignant potential in early passages, but it acquired the ability to grow in soft agar between passage 6 and passage 12; by passage 100, it elicited tumors in nude mice (3,4). HBL-100 cells have been widely used as a model of malignant progression and as a source of normal human mammary epithelial cells (5-9).

Numerous investigations (10-17) in the 1970s concerned a retroviral etiology of human breast cancer. A human breast cancer virus was never isolated, and interest in this possibility waned. New, highly sensitive technology prompted us to re-examine the retrovirus-breast cancer hypothesis.

The product-enhanced reverse transcriptase assay (18) was used to screen three established human breast cancer cell lines, HTB20, HTB121, and HTB126. All of these cell lines were from the American Type Culture Collection (ATCC), Rockville, MD. HBL-100 cells, designated HTB124 passage 27 (ATCC), were used as a control. Only the HTB124 cell supernatant exhibited reverse transcriptase (RT) activity, which was enhanced following cell treatment with iododeoxyuridine and dexamethasone (19) to induce retroviral expression (data not shown). A subsequent standard RT assay (20) showed that the HTB124 supernatant enzyme was 13-fold more active with dT\textsubscript{12-18} · A\textsubscript{n} as template (1.3 × 10\textsuperscript{5} cpm dTMP [i.e., deoxythymidine monophosphate] incorporated/mL culture supernatant) compared with dT\textsubscript{12-18} · dA\textsubscript{n} (1.0 × 10\textsuperscript{6} cpm/mL) and was 20-fold more active with Mg\textsuperscript{2+} cation (1.3 × 10\textsuperscript{5} cpm/mL) compared with Mn\textsuperscript{2+} (6.5 × 10\textsuperscript{5} cpm/mL). These properties are characteristic of RTs of human and non-human primate type D, murine type B, and avian type C retroviruses. The HTB124 supernatant was negative by a laboratory antigen capture assay (Program Resources, Inc., National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD) for human immunodeficiency virus type 1 (HIV-1) and by the Coulter antigen capture assay for simian immunodeficiency virus/HIV-2 p24 (data not shown). Thus, the HTB124 cells were not contaminated with either type of HIV, which were also cultured in the laboratory.

The presence of RT activity in HTB124 cells was intriguing and recalled prior studies on RT in human milk (16). A second vial of ATCC HTB124 passage 27 cells was obtained, and again the cells were RT positive (9.1 × 10\textsuperscript{4} cpm/mL with dT\textsubscript{12-18} · A\textsubscript{n}). Following one passage, cytogenetic and isoenzyme analyses carried out by Applied Genetics Laboratories, Inc. (Melbourne, FL), indicated that the cells were of human origin (data not shown). Transmission electron microscopy showed typical type D retroviral particles in both aliquots of HTB124 cells (Fig. 1). Infectivity of these particles was demonstrated by transmission to T- and B-cell lines via HTB124 cell-free supernatant or coculture with irradiated HTB124 cells. Subsequent RT activity with dT\textsubscript{12-18} · A\textsubscript{n} in supernatants of the T-cell lines CEM and MOLT3 ranged from 2.4 × 10\textsuperscript{2} to 5.2 × 10\textsuperscript{2} cpm/mL; in the B-cell lines NC37 and Daudi, this activity ranged from 7.0 × 10\textsuperscript{4} cpm/mL to 1.8 × 10\textsuperscript{5} cpm/mL. Viral particles were also observed by transmission electron microscopy in CEM cells cocultured with HTB124 cells (Fig. 1).

Since HBL-100 cells are SV40 positive (3), the type D morphology suggested that contamination with a second monkey virus may have occurred. The retrovirus was examined for identity with the following known type D retroviruses: squirrel monkey retrovirus (SMRV) and Mason-Pfizer monkey virus (MPMV). Type B murine mammary tumor virus (MMTV) was also investigated. Southern blot analysis showed that the HTB124 cells were not infected with SMRV (Fig. 2, A). However, MPMV-specific products were amplified by polymerase chain reaction from HTB124 and MPMV DNAs but not from SMRV or MMTV DNAs (Fig. 2, B). Therefore, the retrovirus detected in HTB124 is MPMV or a highly related retrovirus.

Early-, middle-, and late-passage HBL-100 cells from viably frozen stocks separately maintained by one of us (B. C. Giovanella) were MPMV negative (Fig. 2, C). However, all three passages of HBL-100 cells were SV40 positive (Fig. 2, D), suggesting that the MPMV contamination of HTB124 cells was independent of SV40 acquisition.

MPMV was originally isolated from a spontaneous mammary carcinoma of a rhesus monkey (21). While highly related simian type D retroviruses are pathogenic and cause an AIDS-like disease in rhesus monkeys (22,23), there is no evidence that MPMV is etiologically associated with mammary carcinoma. As shown here, the virus plays no role in the malignant progression of HBL-100 cells. However, MPMV readily infects human cells and has been reported in humans (24-27), although its disease-causing potential in humans is unknown. Infection of cells with MPMV could alter biological functions. Early-passage HBL-100 cells possessed estrogen and prolactin receptors and responded to...
hormones with casein production (1,28). HTB124 passage 26 cells, however, lacked both receptors and were unresponsive to hormones (8). Whether contaminating MPMV played a role in down-regulating (i.e., decreasing) these receptors requires further study.

HTB124 cells produce abundant retroviral particles, as judged by the ease of their detection by transmission electron microscopy and their ready transmission. Users of HBL-100 cells should be aware that some cell stocks are MPMV producers.

References


Notes

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