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Expression of the *E. coli Lac Z* gene from a defective HSV-1 vector in various human normal, cancer-prone and tumor cells

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Introducing foreign genetic material into human cells is essential for the elucidation of the function of various human genes and has potential use in the treatment of human diseases by gene therapy. In this study we demonstrate that a defective herpes simplex virus type 1 vector, pHSVlac, can effectively transfer and express the *Escherichia coli Lac Z* gene in a variety of exponential and quiescent human cells. The human cells tested included representative cells derived from cancer-prone patients that presumably have various DNA repair deficiencies.

HSV-1 vector, defective; Host range; Gene therapy; DNA damage, potentially lethal; DNA repair

1. INTRODUCTION

A defective herpes simplex virus type 1 (HSV-1) vector, pHSVlac, was recently developed which contains the *E. coli Lac Z* gene under the control of the HSV-1 Immediate Early 4/5 promoter ([1], see fig.1 for the structure of pHSVlac). This vector was developed to introduce genetic material into postmitotic human or rodent neurons to alter physiology and perform gene therapy on neuronal disorders [1]. For example, Parkinson's disease might be treated by introducing the tyrosine hydroxylase gene into neurons in the striatum [2]. Although HSV-1 has a wide host range, infecting various mammalian and avian cell types [3], the host range of pHSVlac virus has yet to be established. The purposes of this study were to determine its ability to express β-galactosidase in a variety of human cell types, and to examine its effectiveness in growing as well as quiescent cells.

We have investigated the ability of pHSVlac virus to infect and express foreign DNA (i.e., the *E. coli Lac Z* gene) in a variety of human normal and neoplastic cells (including fibroblasts, carcinomas and melanomas) and in human cancer-prone cells (i.e., cells from patients with Fanconi's anemia, Bloom's syndrome, ataxia telangiectasia and xeroderma pigmentosum variant). The isolation and functional elucidation of human genes involved in DNA repair (particularly in DNA repair processes occurring in quiescent human cells) has been hampered by the inability of existing gene transfer methodologies to efficiently transfect human cells thought to be deficient in DNA repair, namely certain cells from cancer-prone patients [4,5]. New means of introducing foreign DNA into these cells are essential to understand the role(s) of genes involved in DNA repair, and the role of DNA damage-inducible genes or gene products which have recently been described [6–8].

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2. MATERIALS AND METHODS

2.1. *Cell culture techniques and viral infections*

Cell designations used in this study are given in table 1; the cells were obtained and characterized previously [6,9]. The properties of specific cancer-prone diseases have been reviewed [4]; characteristics of individual cell types can be obtained from the Human Genetic Mutant Cell Repository 1988/1989 Catalog of Cell Lines (NIH Publication No.89-2011, Oct., 1988). The growth state of U1-Mel and AT2052 cells was monitored by assessing the percentage of [^{3}H]thymidine-labeled nuclei at exponential and plateau-phase under previously decsdribed conditions [6]. All cells were routinely determined to be free of mycoplasma as described [10].

Typical experiments were initiated by plating 5×10^4 viable cells in 35 mm tissue culture dishes, or 1×10^4 viable cells in 24 well tissue culture plates with α-media containing 10% fetal bovine serum, 4 mM glutamine and nonessential amino acids [6]. A correction was made in the number of cells plated for each cell type based upon variations in plating efficiencies (table 1). Cells were grown in a humidified 6% CO_2–94% air atmosphere at 37°C for 24 h. Medium was removed and triplicate cell cultures were infected with pHSVlac virus (grown with HSV-1 strain 17 ts K as helper virus [11]), or with ts K alone, as described [1]. Infections were performed at a multiplicity of infection (m.o.i.) of 0.05 (unless otherwise indicated) in order to achieve a theoretical infection of 5%. ts K was titered using CV-1

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Table 1

Expression of β-galactosidase derived from pHSVlac virus in human cancer-prone, normal and tumor cells

Cell type[a]	Percentage (%) of β-galactosidase-positive cells			
	Experiment 1		Experiment 2	
	pHSVlac virus	ts K virus	pHSVlac virus	ts K virus
I Human normal cells				
GM2936B	1.46 ± 0.04	0.0	1.95 ± 0.68	0.0
GM2907A	1.68 ± 0.03	0.0	3.14 ± 0.22	0.0
AG2603	2.36 ± 0.13	0.0	N.D.	
II Human tumor cells				
HEp-2	1.09 ± 0.01	0.0	1.13 ± 0.18	0.0
HTB-152	2.10 ± 0.41	0.0	2.62 ± 0.84	0.0
U1-Mel				
Exponential	5.84 ± 0.85	0.15 ± 0.003	4.83 ± 0.12	0.08 ± 0.001
Plateau-phase[b]	9.26 ± 0.03	1.35 ± 0.06	7.46 ± 0.31	0.96 ± 0.002
III Human cancer-prone cells				
AT2052				
Exponential	1.12 ± 0.16	0.0	1.95 ± 0.68	0.0
Plateau-phase[b]	9.61 ± 0.75	0.0	N.D.	
BS2548	1.13 ± 0.10	0.0	N.D.	
FA2053	1.18 ± 0.09	0.0	1.21 ± 0.04	0.0
XPV2359	1.82 ± 0.03	0.0	2.11 ± 0.12	0.0
IV Monkey cells				
CV1	2.32 ± 0.16	0.0	2.16 ± 0.8	0.0

[a] Cell designations are: GM2936B and GM2907A, normal nonfetal human fibroblasts; XPV2359 (GM2359), xeroderma pigmentosum variant fibroblasts; AT2052 (GM2052), ataxia telangiectasia fibroblasts; BS2548 (GM2548A), Bloom's syndrome fibroblasts; FA2053 (GM2053), Fanconi's anemia (Pancytopenia) fibroblasts; U1-Mel, malignant human melanoma; HTB-152, metastatic human lung fibrosarcoma; HEp-2, human laryngeal epidermoid carcinoma; AG2603, normal fetal lung fibroblasts; and CV1, monkey kidney fibroblasts. Plating efficiencies were 70–90% for normal and tumor cells and 40–60% for human cancer-prone cells

[b] Cells were infected in triplicate with an m.o.i. of 0.15 for U1-Mel cells and 0.3 for AT2052 cells. Cell nuclei from plateau-phase cultures were stained with 1 μg/ml Hoechst dye 33258 (Calbiochem-Behring Corp., La Jolla, CA) and counted using a UV-microscope to determine total cell numbers

Cells were plated and infected at an m.o.i. of 0.05 (unless otherwise noted) as described in section 2. Triplicate samples were scored in each experiment

cells and pHSVlac was titered using PC12 cells. The titre of pHSVlac virus was 4×10^4 infectious particles/ml of pHSVlac and 5×10^4 plaque forming units (i.e., pfu)/ml of ts K. The titre of ts K alone was 1.0×10^6 pfu/ml. Mock-infections were performed by adding an equivalent volume of α-medium alone to parallel cultures. At 24 h after infection, cultures were fixed with 5% glutaraldehyde and scored for the percentage of cells expressing β-galactosidase activity as described [1,11–13].

3. RESULTS

To investigate the range of infectivity of pHSVlac virus, various human cancer-prone, normal and tumor cells were infected with pHSVlac virus and the number of β-galactosidase-positive cells was determined. The results of two experiments, each completed in triplicate, are shown in table 1. All cell types, including representative cells from cancer-prone patients, showed darkly stained cells representing positive β-galactosidase activity (fig.2) derived from the introduction of the *E. coli Lac Z* gene contained in pHSVlac (fig.1). Both exponential (i.e., >35% [^{3}H]thymidine-labeled nuclei) and plateau-phase arrested (<5% labeled nuclei) U1-Mel or AT2052 cells showed β-galactosidase activity (table 1). pHSVlac virus was also able to express β-galactosidase activity in human neuroblastoma (SK-N-BE(2)) cells and in human glioma (Hs 683) cells derived from the temporal lobe (Geller, A., unpublished results). None of the cultures infected with ts K alone or mock-infected contained β-galactosidase-positive cells, except for U1-Mel cells (both plateau phase and log phase) which displayed a low number of lightly stained positive cells following infection with ts K alone (table 1).

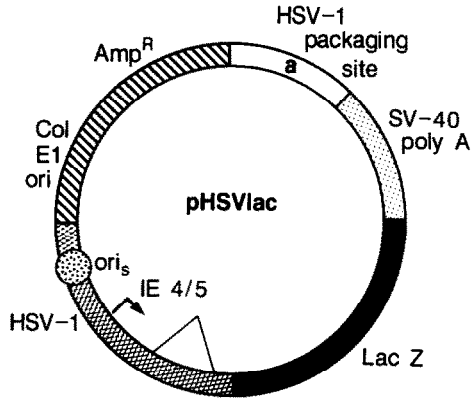

Fig.1. Structure of the defective HSV-1 vector, pHSVlac. The construction of the defective HSV-1 vector, pHSVlac, has been previously described [1]. pHSVlac contains a transcription unit composed of the HSV-1 IE 4/5 early promoter (arrow), the intervening sequence following that promoter (triangle), the *E. coli Lac Z* gene and the SV-40 early region polyadenylation site. To be replicated during a HSV-1 viral infection and subsequently packaged into HSV-1 virus particles, pHSVlac contains an HSV-1 origin of DNA replication (circle) and an HSV-1 packaging site. pHSVlac also contains an ampicillin resistance (amp^R) gene and an *E. coli* origin of DNA replication, which supports the growth of the pHSVlac vector in *E. coli*. Reprinted with permission from Science [1].

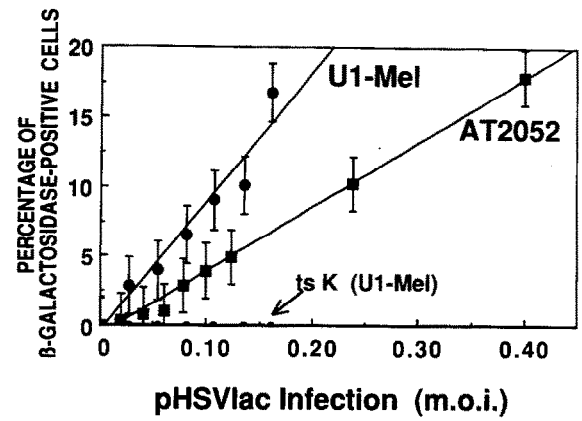

Fig.3. β-Galactosidase activity in U1-Mel and AT2052 cells with increasing amounts of pHSVlac virus. U1-Mel cells (6.0×10^4) or AT2052 cells (1.7×10^4) were infected with 5–80 μl of pHSVlac virus (corresponding to m.o.i.'s of 0.01–0.40) in 24 well tissue culture plates. β-Galactosidase-positive cells were visualized as described in section 2.

AT2052 and U1-Mel cells were chosen to investigate the efficiency of producing β-galactosidase-positive cells following infection with increasing m.o.i of pHSVlac virus. Fig.3 shows a linear relationship bet-

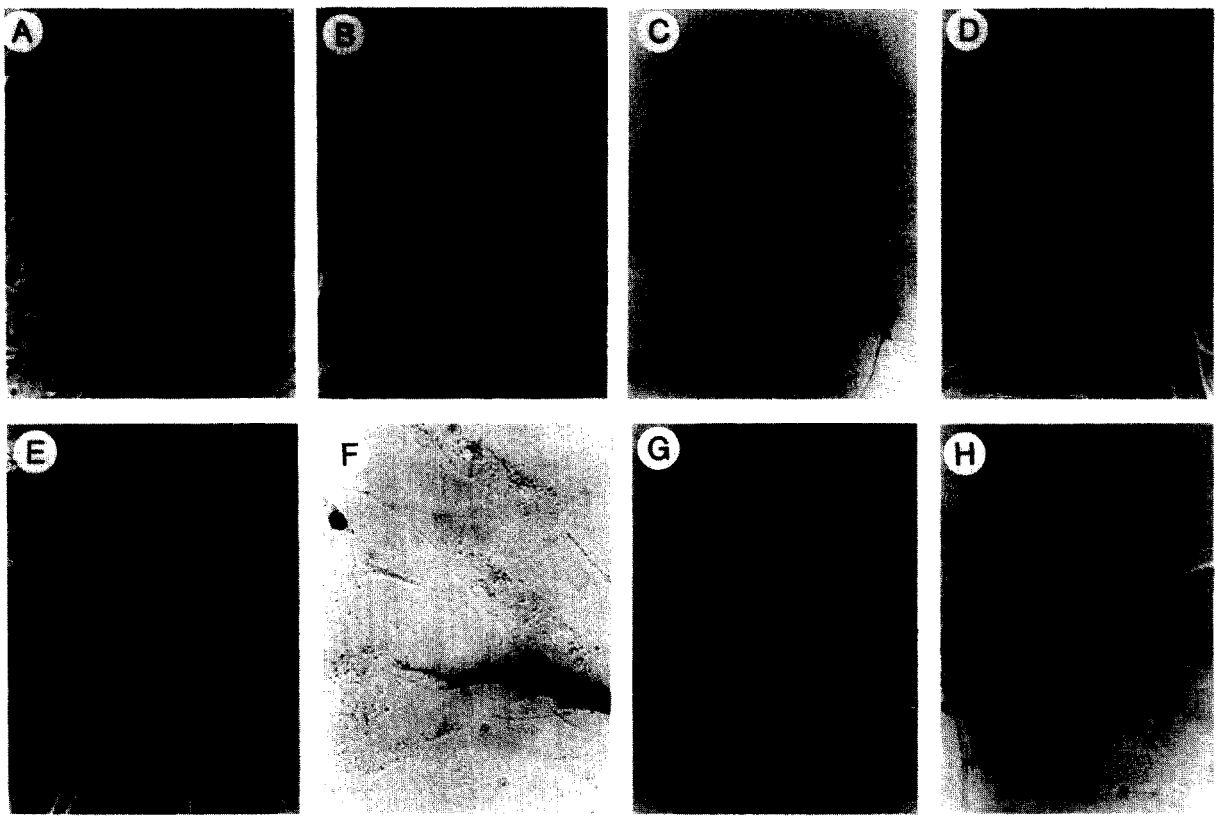

Fig.2. pHSVlac-mediated expression of β-galactosidase activity in human normal, tumor and cancer-prone cells. Human cells were infected and scored for β-galactosidase activity (appearing as darkly stained cells in photomicrographs A–H) as described in table 1 and section 2. Human cells shown are: (A) U1-Mel (10 ×); (B) U1-Mel (32 ×); (C) GM2936B (32 ×); (D) AG2603 (10 ×); (E) AT2052 (32 ×); (F) BS2548 (32 ×); (G) FA2053 (32 ×); and (H) XPV2359 (32 ×).

ween increasing amounts of pHSVlac virus and the percentage of β-galactosidase-positive U1-Mel cells. More than 18% of U1-Mel cells exhibited β-galactosidase activity at a m.o.i. of 0.21. Under identical conditions, AT2052 cells showed a significantly lower, but linear production of β-galactosidase-positive cells; at an m.o.i. of 0.25 only 10% of the cells were β-galactosidase-positive. No cell death was noted at any of m.o.i. of pHSVlac virus used.

4. DISCUSSION

Human cells of various types can be infected at a high efficiency (table 1) with foreign DNA carried by pHSVlac, a defective HSV-1 viral vector. pHSVlac virus infected exponential as well as quiescent human cells (table 1), and efficiently expressed its transcription unit (i.e., the *E. coli Lac Z* gene under the control of the HSV-1 Immediate Early 4/5 promoter) in epithelial (i.e., HEp-2), fibroblast (e.g., ataxia telangiectasia) and neural crest derivative (i.e., U1-Mel melanoma) human cells. Stable expression, for up to 7 days, was observed in various quiescent normal, tumor and cancer-prone human cells (data not shown) and for up to two weeks in postmitotic rat neurons [1]. We have been unable, however, to obtain a significant frequency of exponential human cells which stably (for >6 cell divisions) express β-galactosidase using pHSVlac (data not shown). The insertion of a selectable marker (e.g., neomycin-resistance) into pHSVlac may allow us to establish stable transfectants of mitotic cells.

The ability of pHSVlac virus to deliver genes into human cells has the potential to both study and treat human diseases. New and improved modes of gene transfer are needed for the elucidation of the role(s) of various induced [6–8] and/or constitutive genes involved in processes such as DNA repair, which is carried out in quiescent human cells. This vector may be useful for the elucidation of genes involved in potentially lethal DNA damage repair (PLDR) following X-irradiation [6]. At present, cancer-prone cells represent the only potentially DNA repair-deficient human cells, and their resistance to transfection has hindered progress in isolating and characterizing such human DNA repair genes [3–5]. We have demonstrated that pHSVlac can efficiently transfer and express foreign DNA into these cells (table 1, figs 2 and 3). Because of the high infection frequencies (table 1) and large insertion capacity, HSV-1 vectors may provide a useful tool for elucidating human gene(s) involved in repairing various types of DNA damage.

HSV-1 vectors carrying genes other than *Lac Z* may also be used in human gene therapy to treat neuronal diseases. For example, with such vectors one may be able to deliver the tyrosine hydroxylase gene into appropriate neurons in the brain to increase dopamine production to treat Parkinson's disease [2].

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