Efficient retroviral-mediated gene transfer into human B lymphoblastoid cells expressing mouse ecotropic viral receptor

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Human B lymphoblastoid cell lines have been widely used as recipients to study the function of defined oncogenes in B cell growth, differentiation and tumorigenicity (1, 2). A major limitation in these analyses is the low efficiency with which exogenous genes can be transduced into such cells even by using amphotropic retroviral vectors. This necessitates the cotransfer of a dominant selectable drug marker gene together with a protracted period of cellular selection (2), making it difficult to correlate changes in cell characteristics with expression of the exogenous gene in question. We report here that introduction of the gene encoding the mouse ecotropic retroviral receptor into human B lymphoblastoid cells renders them highly susceptible to infection with retroviral vectors of ecotropic host range and obviates the need to use selectable markers.

A 2.3 Kb *Bam*HI–*Eco*RI cDNA fragment encoding the mouse ecotropic receptor from the plasmid pJET (a gift from Drs L.Albritton and J.Cunningham) (3) was inserted into the vector pM5neo (4) (Figure 1) and transfected into cocultures of retroviral packaging cell lines ψ 2 and PA317 to generate high titre amphotropic virus, essentially as described by Kozak and Kabat (5). This virus was introduced into 2×10^6 SKW6.4 human B lymphoblastoid cells (6) by cocultivation with 10^6 virus-producing PA317 cells in RPMI medium containing 10% fetal calf serum (FCS) for 3 days followed by selection with G418 (1 mg/ml) for 1 week. B lymphoblastoid cells expressing functional ecotropic virus receptor were further selected by infecting 5×10^6 cells with 5 ml of filtered supernatant from GPE(BP)6 cells (7) releasing ecotropic virus (10^4 infectious units — IU/ml) conferring resistance to the drug puromycin. Following selection in 2.5 μ g/ml puromycin for one week, the susceptibility of the resulting cells to infection with ecotropic virus was monitored using virus (5×10^4 IU/ml) from ψ 2 BAG cells (8) expressing the β -galactosidase gene. As shown in Figure 2, *in situ* staining for β -galactosidase activity revealed that a majority of the cell population became stably infected after a 1–2 day cocultivation with ψ 2 BAG cells (typically 60–100%) whilst control SKW6.4 cells were less than 1% positive. Retroviral vectors of ecotropic host range expressing a convenient marker β -galactosidase gene can therefore be used to efficiently transduce genes into such modified human B lymphoblastoid cells, a procedure which in addition minimizes potential biohazards associated with manipulating amphotropic virus expressing oncogenic sequences.

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Figure 1. Structure of the pM5neo vector harbouring the mouse ecotropic viral receptor gene. LTR: retroviral long terminal repeat; B: *Bam*HI; E: *Eco*RI; SD: splice donor; SA: splice acceptor. The vector construct was cloned into pUC18 (not shown).

Figure 2. *In situ* staining for β -galactosidase activity following infection of lymphoblastoid cells with ecotropic virus from ψ 2 BAG cells. 10^6 cells from the parental SKW6.4 (A) or from a clone of SKW6.4 cells expressing ecotropic receptor (B) were cocultivated for 2 days with 10^6 ψ 2 BAG cells in RPMI medium containing 10% FCS. Following a further 2 days of growth, B lymphoblastoid cells were monitored for β -galactosidase activity *in situ* using 5-bromo-4-chloro-3-indolyl- β -D-galactoside substrate, essentially as described by Sanes *et al.* (9). Photographs were taken at 200 $\times$ magnification.