Application note

Transfection of a 231 kb Bacterial Artificial Chromosome Vector

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GenePORTER™ Transfection Reagent

Introduction

Transfection of bacterial artificial chromosome (BAC) clones, which are large vectors commonly in excess of 100 kb, remains very difficult compared to transfection of common plasmid vectors, which are typically < 15 kb. In this article, we demonstrate the efficient transfection of Chinese hamster mutant V-C8 cells with a bacterial artificial chromosome (BAC) containing the breast cancer susceptibility gene, *Brca2*, using the GenePORTER™ transfection reagent. This experiment was performed to verify if V-C8 cells are defective in *Brca2* and to determine if hypersensitivity in response to various DNA damaging agents is caused by Brca2 deficiency (1).

Materials and Methods

Cell line.

V-C8 is a Chinese hamster mutant cell that represents the XRCC11 complementation group among X-ray-sensitive rodent cell mutants. It is extremely sensitive to various DNA-damaging agents (2, 3, 4). The high level of spontaneous and cross-linked induced chromosomal aberrations manifested by V-C8 cells indicates a possible defect in DNA repair. Indeed, V-C8 cells have an impaired capacity to repair double-stranded breaks after irradiation (3). A key player in double-stranded break repair through homologous recombination is the Rad 51 protein (5), a homolog of the E. coli RecA protein. Impaired formation of Rad51 foci, in response to DNA damage has been demonstrated in mammalian Brca1 or Brca2 defective cells, as well as in hamster cells defective in Rad51 paralogs.

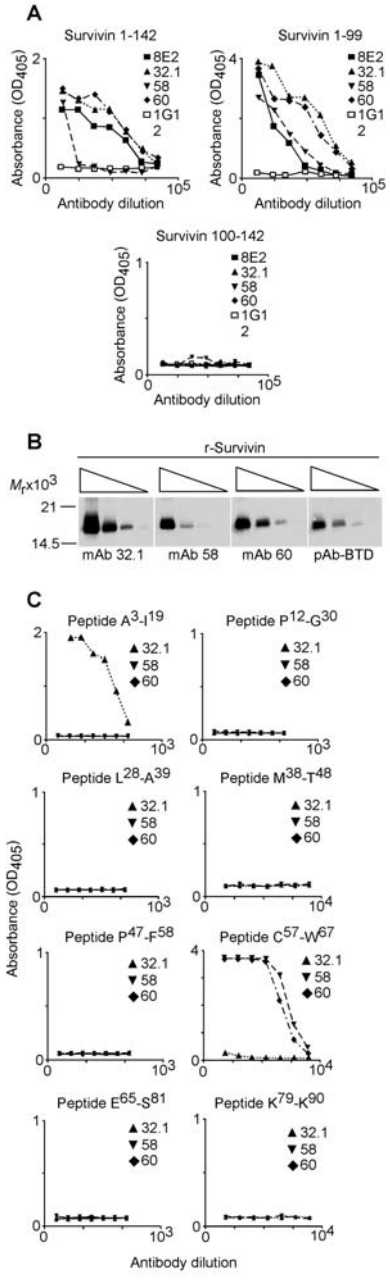
BAC vector

pBAC421-Neo is a bacterial artificial chromosome vector based on the 11.5 kb pBACe3.6 vector. pBAC421-Neo contains a 220-kb genomic sequence consisting of the full-length 60 kb murine *Brca2* gene, 70 kb of upstream sequences, and 90 kb of downstream sequences. This BAC has been used to completely rescue the embryonic lethality associated with the Brca2 mutation in mice (S. Swaminathan and S. K. Sharan, unpublished data).

Transfection

Transfection of pBAC421-Neo was performed using the GenePORTER transfection reagent according to the manufacturer's protocol. Briefly, V-C8 cells were incubated overnight so they were 60-90% confluent on the day of transfection. pBAC421-Neo DNA was mixed with GenePORTER transfection reagent in serum-free medium and allowed to incubate for 10-45 minutes. The DNA/GenePORTER complexes were added to the cells and allowed to incubate in serum-free medium at 37˚C. After 3-5 hours, one volume of medium containing

Figure 1. Cell Survival After Exposure



20% FCS was added to the transfection, and the mix was allowed to incubate overnight under 5-10% CO_2 at 37˚C. Cells were assayed for clonogenic survival after 24-72 hours.

Clonogenic Survival Assays

To determine clonogenic survival of V-C8 cells after transfection of pBAC421-Neo, cell cultures were transferred to 10 cm dishes and exposed either to X rays, or to the DNA damaging agents mitomycin C (MMC) and methanesulfonate (MMS). After treatment, the cells were rinsed with 0.9% NaCl, stained with 0.25% methylene blue, and visible colonies were counted.

Results

Using the clonogenic survival assay described, we observed rescue of clonogenic survival in the pBAC421-Neo transfected V-C8 cells compared to non-transfected cells (Figure 1). This confirmed the results of a separate experiment in which a single human chromosome 13 providing the *Brca2* gene (1) was transferred into V-C8 cells by microcell-mediated chromosome transfer (1). The sensitivities of V-C8 to X rays, MMC, MMS, and UV light (data not shown) were largely complemented by human chromosome 13 (Figure 1).

Discussion

In conclusion, the Chinese hamster cell mutant, V-C8, is defective in the breast cancer susceptibility gene *Brca2*. Transfection of a BAC containing the *Brca2* gene into V-C8 cells allows for rescue of resistance to DNA damaging agents and clonogenic survival. GenePORTER transfection reagent effectively delivers BAC's into these cells, allowing the gene of interest to be expressed and the effects of the expressed protein to be studied.

References

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