APOPTOSIS GENE HUNTING USING RETROVIRAL EXPRESSION CLONING

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INTRODUCTION. cDNA expression cloning is a powerful technique for the isolation of genes, which confer selectable phenotypes on specific cell types. This strategy has been used successfully to identify potential oncogenes (1), genes capable of suppressing cytokine signal transduction (2) and genes encoding cell surface antigens (3). We have utilised a functional expression cloning strategy in conjunction with a retroviral vector, pRUF*neo*, to search for important regulatory genes involved in apoptosis. This approach has the advantage that it assumes no prior involvement in apoptosis or makes any assumptions concerning nucleotide sequence or protein-protein interactions.

METHOD. BAF-3 cells, a murine interleukin (IL)-3-dependent cell line of pro-B lymphocytes, were infected with the retroviral vector, pRUF*neo*, into which a cDNA library from the factor dependent cell line FDC-P1 had been inserted (4). IL-3 deprivation of retrovirally infected BAF-3 cells was used to identify cDNAs that conferred resistance to IL-3 withdrawal-induced apoptosis, by plating in soft agar in the presence of IL-3 and geneticin. Genomic DNA from IL-3 resistant clones was isolated and amplified by PCR using pRUF*neo* vector primers to recover cDNA inserts, which were subsequently identified by sequencing.

RESULTS. Several IL-3 resistant clones were isolated (Fig. 1) and cDNA inserts were recovered by PCR (Fig. 2). Sequence analysis of these inserts revealed a 3' untranslated portion of the vacuolar ATPase subunit E gene, ribosomal protein L17 and saposin, in addition to several putatively novel sequences. Functional analysis of the degree of apoptosis resistance conferred by these cDNA inserts will be presented.

A. Parental BAF-3 cells

(i) No G418

(ii) 1 mg/ml G418

B. Retrovirally infected BAF-3 cells

(i) No G418

(ii) 1 mg/ml G418

Figure 1

Figure 2

DISCUSSION. We have shown that cDNA expression cloning utilising a retroviral vector system can be successfully used to isolate potentially novel apoptosis controlling genes, as well as known genes whose role in IL-3 withdrawal-induced apoptosis has not previously been demonstrated.

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