Construction and application of L929 cell model expressing human bcl-2 protein

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Abstract Recombinant eucaryotic expression vector pLXSN/s-bcl-2 has been constructed by cloning human *bcl-2* cDNA containing the full-length open reading frame into the vector pLXSN in sense orientation, and a mammalian cell model expressing human bcl-2 protein has been established by electroporating the recombinant vector into mouse L929 cells. bcl-2 expression in L929 cells has no effect on the cell growth and survival under normal culture conditions, but it can enhance the survival of the cell in the challenge of some apoptosis-inducing stimuli, including tumor necrosis factor α(TNF α) and staurosporine (STS).

Keywords: **bcl-2, cell model, L929 cell line, apoptosis.**

THE *bcl-2* is an important apoptosis-regulating gene, which was involved in many physiologic functions and pathogenesis. Functional analysis of *bcl-2* and study on the regulation of the gene expression will offer help for preventing and treating some associated diseases, especially some *bcl-2*-associated tumors[1,2]. However, the mechanism of *bcl-2* action remains unknown. It is very useful to establish a *bcl-2*-expressing cell model for evaluating *bcl-2* actions. In this note, a mammalian cell model stably expressing human Bcl-2 protein was established by transferring human *bcl-2* cDNA into mouse L929 cell, and the functions of *bcl-2* gene in the model cell were explored. The results enrich the data of apoptosis regulated by *bcl-2* and may pave a way for further study on the mechanism of *bcl-2* action and the interactions between *bcl-2* and other genes.

1 Materials and methods

Plasmid pB4[3] was digested with *Eco*R I and electrophoresed on low melting agarose gel. The 0.91 kb human *bcl-2* cDNA was isolated from the gel and ligated with the vector pLXSN linearized by *Eco*R I. Competent *E. coli* MC1061 was transformed with the ligated sample. The recombinant vector pLXSN/s-bcl2 in which *bcl-2* cDNA was inserted in sense orientation was identified by using cracking gel analysis and mapping with *Eco*R I and *Bam*H I enzymes. pLXSN/s-bcl2 and control vector pLXSN were electroporated into mouse L929 cells respectively and the G418-resistant clones were selected, which were named L929/s-bcl2 and L929/neo cells respectively. Lysates of L929/s-bcl2, L929/neo and L929 cells were prepared by the method described in ref. [4], and the bcl-2 protein was detected by Western

blot analysis. After having been cultured continuously for 20 generations in the presence or absence of G418, the G418-resistant characterization and the level of bcl-2 expression in L929/s-bcl2 and L929/neo cells were evaluated.

To inquire into the effect of bcl-2 expression on cell growth and survival under normal conditions, L929/s-bcl2, L929/neo and L929 cells were inoculated at 4×10^4 cells/well in 24-well plates and cultured in complete medium. Then at 24-h intervals (over a period of 4 d), cells in partial wells were harvested and counted in double samples, and the cell viability was determined visually by Trypan blue dye exclusion. The cell number and viability of the 3 kinds of cells were compared.

TNF α cytotoxicity assay was carried out with two methods. (ⅰ) Cells were inoculated at 1.2×10^4 cells/well in 96-well microtitre plates and cultured overnight, different dose of recombinant human TNF α (rhTNFα) and actinomycin D at terminal concentration 1.5 μg/mL were added. After 20 h, cell viability assay with MTT was done and the absorbance at wavelength 595 nm (A_{595}) was determined. The cell viability was defined as the ratio (A_{595} of the tested sample/A_{595} of the control cell) × 100%. (ⅱ) Cells were inoculated at 1.2×10^4 cells/well and cultured overnight, rhTNFα was added at terminal concentration 100 U/mL. At appropriate times, MTT assays were done in the absence of actinomycin D. The percent age of inhibited cells was defined as the ratio $(A_c - A_t)/A_c \times 100\%$, where A_c is the A_{595} of control cell and A_t is the A_{595} of the tested sample. In the above two methods, RPMI-1640 without phenol red was used, and the cells untreated with rhTNFα were as controls, and the MTT assay was done in triplicate.

To observe the effect of STS challenge, the cells were inoculated in triplicate at 1.2×10^4 cells/well in 96-well plates in RPMI-1640, without phenol red, plus 1.2 μmol/L STS. At 8-h intervals, MTT assay was done and the cell viability was defined as the ratio (A_{595} of the tested sample/A_{595} of the control cell) × 100%. The cells untreated with STS were as control.

2 Results and discussion

Western blot (fig. 1) shows that L929/s-Bcl2 cell could express 25—26 ku human Bcl-2 protein, while L929/neo and L929 cells could not. After having been cultured continuously for 20 generations in the absence of G418, both L929/s-bcl2 and L929/neo cells still kept the ability to resist G418. In the presence or absence of G418, the levels of bcl-2 expression in L929/s-bcl2 cells had no significant difference during that period, which suggested that human Bcl-2 protein was expressed stably in the model cell.

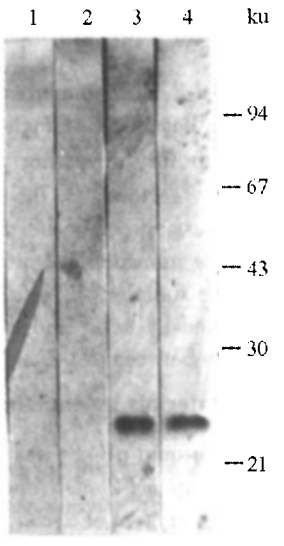

Fig. 1. Western blot analysis of the expression of Bcl-2 protein in L929 cells. 1, L929 cell; 2, L929/neo cell; 3, 4, L929/s-bcl2 cells.

In normal culture conditions, the cell number and viability of L929/s-bcl2 cell had no significant difference compared with L929/neo or L929 cells at given times, which suggested that human bcl-2 expression in L929 cell may have no effect on the cell normal growth and survival. Recently, Miyazaki *et al.*[5] found that *bcl-2* was involved in IL-2 receptor-mediated signaling pathway, and could cooperate with *c-myc* or $P56^{lck}$ to promote cell proliferation, which indicated that *bcl-2*, cooperating with some other genes, may regulate proliferation of some cells to some extent. The establishment of the L929/s-bcl2 cell model may provide help for such studies.

As shown in fig. 2, expression of human Bcl-2 protein could enhance the survival of mouse L929 cells in TNFα-mediated cytotoxicity. The effect of TNFα varies depending on the conditions. For example, TNFα can induce apoptosis and inhibit bcl-2 expression in some cases[6], while in other cases TNFα can up-regulate bcl-2 expression and suppress apoptosis[7]. This may result from the heterogeneity of the target cells and the difference in intracellular signaling pathways. TNFα can not only induce apoptosis, but also activate NF-kB. However the two pathways are independent, diverging early in the TNF α signaling cascade[8]. Activation of NF-kB can suppress

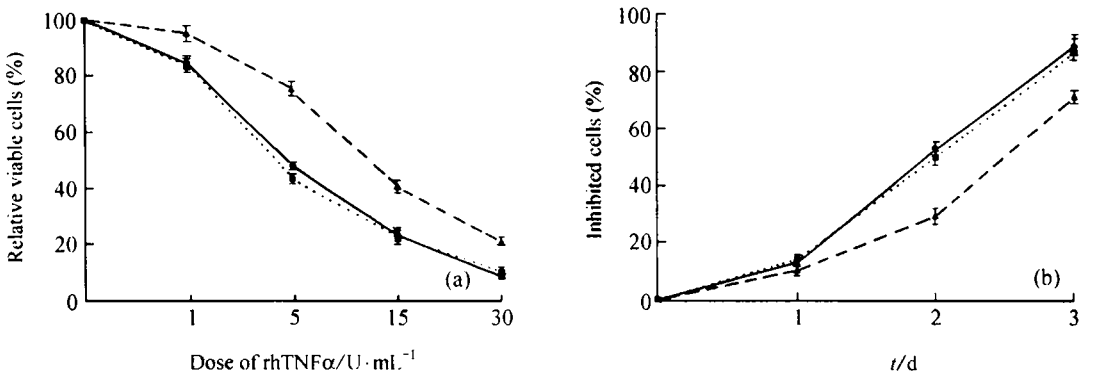

Fig. 2. rhTNFα challenge of the cells. (a) Cells were treated with different doses of rhTNFα in the presence of actinomycin D, and the cell viability was determined by MTT assay and expressed as a control relative to untreated cells; (b) cells were cultured for various times with 100 U/ml rhTNFα in the absence of actinomycin D, and the number of viable cells was determined by MTT assay and the percentage of inhibited cells was defined as described in sec. 1. ···▲···, L929/s-bcl2 cells; ···■···, L929/neo cells; —●—, L929 cells. Points, mean; bars, standard deviation (SD).

TNFα-induced apoptosis[9]. Mouse L929 cell is sensitive to TNFα, so the establishment of the L929/s-bcl2 cell model may be beneficial to the study of the effect of bcl-2 expression on TNF α signaling cascade.

As shown in fig. 3, expression of human Bcl-2 protein could enhance the survival of L929 cells in STS challenge. STS is a protein kinase inhibitor and can induce many cell types in culture to undergo apoptosis[10], perhaps because it blocks the intracellular signaling pathways activated by the extracellular survival factors that many cells require to live. However it is not clear how the effect of STS was suppressed by *bcl-2*. The construction of the mammalian cell model expressing human Bcl-2 protein may pave a way for elucidating the influence of *bcl-2* on STS action.

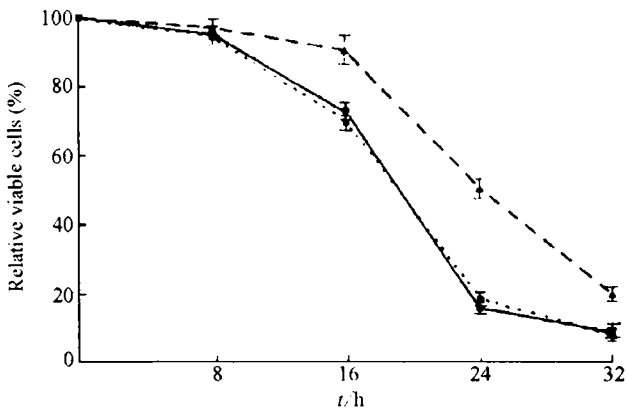

Fig. 3. STS treatment of the cells. Cells were cultured for various times with 1.2 μmol/L STS, anid the cell viability was determined by MTT assay and expressed as a control relative to untreated cells. ···▲···, L929/s-bcl2 cells; ···■···, L929/neo cells; —●—, L929 cells. Points, Mean; bars, SD.

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