Using Bcl-xL anti-apoptotic protein for altering target cell apoptosis

Sultan Gülçe İz¹ ⊠ · Beste Çalımlıoğlu¹.² · Saime İsmet Deliloğlu Gürhan¹

- 1 Ege University, Engineering Faculty, Department of Bioengineering, Bornova, Izmir, Turkey
- 2 Istanbul Medeniyet University, Engineering and Architecture Faculty, Department of Bioengineering, Goztepe, Istanbul, Turkey

Corresponding author: sultangulce@gmail.com
Received April 10, 2012 / Accepted July 8, 2012
Published online: September 15, 2012
© 2012 by Pontificia Universidad Católica de Valparaíso, Chile

Abstract

Background: Altering target cell apoptosis is one of the challenging ideas of biotechnological applications. There are several applications of over expressing Bcl-xL anti-apoptotic protein from recombinant protein production to DNA vaccination strategies. The aim of the present study is to evaluate the anti-apoptotic efficacy of Bcl-xL expressing dual promoter plasmid system as a candidate to be used for recombinant protein production and DNA vaccination approaches. For this purpose, BclxL anti-apoptotic protein gene was inserted in a dual expressing vector system in frame with EGFP (enhanced green fluorescence protein) after IRES (internal ribosomal site). The plasmid has a multiple cloning site after CMV (cytomegalovirus promoter) left empty to be inserted a biopharmaceutical protein gene region or DNA vaccine antigens. Results: In order to determine the anti-apoptotic efficacy of Bcl-xL inserted dual expressing vector, BHK-21 cells were transfected both with this plasmid and empty vector as control. Apoptosis was stimulated by several apoptosis inducing agents and serum deprivation in the transfected cells for 48 hrs. Cells expressing Bcl-xL protein in frame with EGFP were determined by flow cytometry as an indicator of cell viability. Additionally, apoptosis were determined by intracellular cleaved Casp 3 staining in Bcl-xL expressing EGFP positive cells. The dual expression plasmid bearing Bcl-xL anti-apoptotic protein prolonged the cell survival rate and protected cells from apoptosis upon apoptosis induction by doxorubicin and camptothecin in which the anti-apoptotic efficacies are inhibited through over expressing of Bcl-xL. pIRES2EGFP/Bcl-xL transfected cell ratio was significantly higher compared to empty vector transfected cells (P < 0.001). In contrast, apoptotic cell ratio was significantly lower in pIRES2EGFP/Bcl-xL transfected cell population compared to empty vector transfected cells (P < 0.001). Conclusion: In conclusion, it was shown that in vitro transient expression of Bcl-xL efficiently inhibited apoptosis induced by serum deprivation, doxorubicin and camptothecin. Thus, the dual expression plasmid bearing Bcl-xL anti-apoptotic protein could be a good candidate for recombinant protein production and DNA vaccination applications.

Keywords: animal cell biotechnology, apoptosis, Bcl-xL anti-apoptotic protein

INTRODUCTION

Altering target cell apoptosis is one of the challenging ideas of biotechnological applications. Over expression of anti-apoptotic proteins in biopharmaceutical protein production using animal cells as host increases the yields (Al-Rubeai and Singh, 1998; Kim and Lee, 2000; Kim et al. 2009; Han et al. 2011; Kim et al. 2011) as well as enhances the host immune response elicited against the vaccine antigen in DNA vaccination (Kim et al. 2004; Kim et al. 2005; Huang et al. 2007). In addition, Bcl-xL over expression efficiently inhibits neuronal cell death (Blömer et al. 1998), protects cardiac tissue from ischemia (Huang et al. 2003; Huang et al. 2005), inhibits dendritic cell death in melanoma (Kim et al. 2005) and also inhibits lymphocyte cell death in SARS (Yang et al. 2005). In contrast, most of the

cancer therapeutics must induce apoptosis to kill the cancer cells which become resistant to apoptosis through Bcl-xL over expression (Brunelle and Zhang, 2010). Another application of using Bcl-xL antiapoptotic protein is the expressing membrane associated proteins fused with Bcl-xL can be purified easily and cost effectively (Thai et al. 2005).

During biopharmaceutical production due to the oxygen, nutrient limitations, exposure to shear stress, using serum free medium and viral infections can result in apoptosis induction (Al-Rubeai and Singh, 1998; Mastrangelo et al. 2000; Figueroa et al. 2004). To increase the productivity of the recombinant protein, apoptosis is suppressed by anti apoptotic proteins like Bcl-2 and Bcl-xL (Al-Rubeai and Singh, 1998; Mastrangelo et al. 2000) in Chinese hamster ovary (CHO) and Baby hamster kidney (BHK) cells which are commercially valuable cells and mostly used as host for recombinant protein production (Andersen and Krummen, 2002; Figueroa et al. 2003; Figueroa et al. 2004; Kim et al. 2009). Over expression of Bcl-2 and Bcl-xL results in similar protection in both CHO and BHK cells under various stress conditions like Sindbis virus infection, serum and glucose deprivation and ammonia toxicity (Figueroa et al. 2003).

The consequences of Bcl-xL over expression have been widely studied from different aspects. A recent study has demonstrated that Bcl-xL over expression not only inhibits apoptosis but also regulates cell proliferation and survival (Baik and Lee, 2009). Another anti-apoptotic protein Aven, acts in combination with Bcl-xL to enhance the function of Bcl-xL and shows protection against Sindbis virus infection (Figueroa et al. 2004) through blocking DNA damage-induced apoptosis by stabilizing Bcl-xL (Kutuk et al. 2010), XIAP (X-linked inhibitor of apoptosis protein) delays serum deprived apoptosis in CHO cells (Liew et al. 2010).

Apart from apoptosis, autophagy can also occur through nutrient deprivation (Zustiak et al. 2008; Kim et al. 2009). Bcl-xL over expression inhibits both apoptosis and autophagy in erythropoietin producing CHO cell culture (Kim et al. 2009). Also, the over expression of Bcl-xL delays autophagy and apoptosis in hyper osmotic CHO cell cultures resulting from NaCl addition in both batch culture and nutrient supplementation in a fed-batch cultures and also increased erythropoietin production (Han et al. 2011). It has been shown that Bcl-xL over expression both inhibits dimethyl sulfoxide (DMSO) induced and Na-butirat induced apoptosis (Kim and Lee, 2000; Kim et al. 2011) which are used to increase the specific productivity of foreign proteins in mammalian cells.

The aim of the present study is to determine the anti-apoptotic efficacy of the Bcl-xL expressing dual promoter plasmid designed to be used in biotechnological applications like recombinant protein production through transient gene expression and DNA vaccine applications for long-term specific antigen expression after in vivo transfection against several infectious agents or several types of cancer. The over expressing of Bcl-xL using CHO cells enhanced the transient gene expression levels of a therapeutic human fusion protein (Majors et al. 2008); long term antigen expression in DNA vaccine transfected cells expressing Bcl-xL increased the life span of the DNA vaccine transfected cells and antigen specific CD8⁺ T cell responses (Kim et al. 2004; Kim et al. 2005; Huang et al. 2007).

For this purpose, apoptosis were induced by doxorubicin, camptothecin and by serum deprivation in pIRES2EGFP-Bcl-xL plasmid transfected BHK-21 cells. Apoptosis can be induced by different ways due to intrinsic and extrinsic pathways, as well as several chemical agents. In most of the studies apoptosis was induced by serum deprivation (Lindenboim et al. 1995; Akiyama et al. 2002). Doxorubicin is an intercalating agent and camptothecin is a topoisomerase I inhibitor (Kaufmann and Earnshaw, 2000) used to induce apoptosis in the treatments against several types of cancer including breast and colon in which the efficacies are inhibited through over expressing of Bcl-xL anti apoptotic protein (Fiebig et al. 2006; Park et al. 2007; Brunelle and Zhang, 2010).

MATERIALS AND METHODS

Constructing Bcl-xL bearing dual expression plasmids

A dual promoter pIRES2EGFP (Clontech, USA) vector was used for the plasmid construction.

Briefly, Bcl-xL anti-apoptotic protein gene (GeneBank accession no: AAC53459.1) was amplified from the total RNA of B6 MC57 mice kidney by RT-PCR with the following primers, 5'-

CCACAACCATGGTGTCTCAGAGCAACCGGGAGC-3' (33 nt, forward primer, contains Bst-XI restriction site), 5'-CCATGGTTGTGGCCTTCCGACTGAAGAGTGAGCCC-3' (35 nt, reverse primer, contains Bst-XI restriction site). Thereafter, Bcl-xL gene was inserted into pGEMT-Easy (Promega, USA) vector system according to the manufacturer's protocol. Thereafter, pIRES2EGFP and pGEMT/Bcl-xL vectors were then digested with Bst-XI and digested Bcl-xL was inserted into pIRES2EGFP to obtain pIRES2EGFP/Bcl-xL in frame with EGFP. The schematic presentation of the Bcl-xL bearing dual expression vector is shown on Figure 1. The orientation and the sequence of inserts and final DNA vaccine were determined by automatic sequencing using the ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and the BioEdit genetic analyzer.

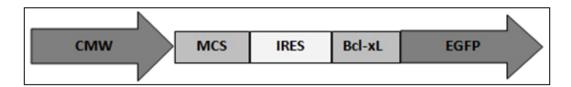


Fig. 1 Schematic presentation of the Bcl-xL bearing dual expression plasmid. CMV, immediate early *promoter* of *cytomegalovirus*; MCS, molecular cloning site for inserting recombinant protein gene regions or DNA vaccine gene regions; IRES, internal ribosomal entry site; Bcl-xL antiapoptotic protein expressed in frame with enhanced green fluorescent protein, EGFP.

Determining IC₅₀ values of doxorubicin and campothesine

BHK-21 cells were maintained in Minimum Essential Medium (MEM) (Gibco, USA) supplemented with 10% FBS (HyClone, USA) 2 mM L-glutamine (Gibco, USA), 1 x penicillin-streptomycine solution (100 U/mL penicillin, 100 μ g/mL streptomycine, Sigma, USA), 0.1 mM non-essential aminoacids (Gibco, USA), 1 mM sodium pyruvate (Gibco, USA) in a humidified atmosphere of 5% CO₂ and 95% air at 37°C.

To determine the IC $_{50}$ values of apoptosis inducing agents several concentrations of doxorubicin (ranging from 0.25 µg/mL to 10 µg/mL) and campothesine (ranging from 12.5 ng/mL to 200 ng/mL) were used. BHK-21 cells were seeded on 96 well plates at a concentration of 6 x 10^4 cells/mL after overnight incubation and treated with different concentrations of doxorubicin and campothesine for 48 hrs. Cell viability and IC $_{50}$ values were determined by MTT assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product. After discarding the exposure medium, 0.5 mg/mL of MTT was added to each well and incubated at 37°C, and 5% CO $_2$ for 4 hrs. After that, 200 µL dimethyl sulphoxide (DMSO) was added to each well to dissolve the formazan salts. The absorbance was immediately determined at 570 nm using a single beam UV-visible spectrophotometer (Molecular Devices, USA).

Bcl-xL expression upon transient transfection

BHK-21 cells (1 x 10^4 cell/mL) were incubated in 24 well tissue culture plates overnight prior to *in vitro* transfection experiments to reach approximately 80-90% confluency at the time of transfection. Cells were transfected using lipofectamine-2000 (Invitrogen, USA) following the manufacturer's protocol with 0.5 μ g DNA/2 μ L lipofectamine 2000 (Invitrogen, USA) complex in serum free Opti-MEM (Invitrogen, USA) medium with empty vector (PIRES2EGFP) and Bcl-xL bearing plasmid (pIRES2EGFP-Bcl-xL) for 4 hrs. Subsequently, Opti-MEM containing DNA-lipofectamine complex were discarded and MEM cell maintenance medium described above were added. After the transfection, cells were incubated for 17-20 hrs prior to treatment with IC50 of apoptosis inducing agents for a following 48 hrs.

Measurement of apoptosis after treatment with apoptosis inducing agents

Upon 48 hrs treatment with apoptosis inducing agents the cells expressing EGFP, transfected with empty vector (PIRES2EGFP) and Bcl-xL bearing plasmid (pIRES2EGFP-Bcl-xL) were determined by flow cytometry. Apoptotic cells were also measured by intracellular staining of cleaved caspase-3 by flow cytometry in the EGFP expressing transfected cells. Briefly, cells were fixed and permeabilized by

Cytofix/Cytoperm fixation and permeabilization solution (BD Biosciences, USA). Cells were stained with anti-cleaved Casp3 antibody (Cell Signaling, USA) and alexa flour 647 R-phycoerythrin labelled IgG secondary antibody was used for flow cytometric analysis. For each group 10000 cells were analyzed by CellQuest analyzing programme using BD FACS Aria flow cytometer (USA).

Statistical analysis

All assays were repeated at least three times to ensure reproducibility and three replicates of each group were performed in each test. The significance of difference between apoptosis inducing agents transfected with pIRES2EGFP and pIRES2EGFP/Bcl-xL plasmid were analyzed by one way analysis of variance (ANOVA) followed by Benferroni's post-hoc comparison by Prism 5.0 (Graphpad, USA).

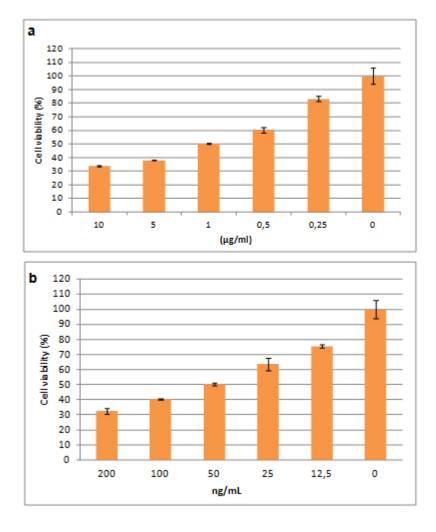


Fig. 2 (a) IC $_{50}$ value of doxorubicin on BHK-21 cells is 1 μ g/mL. (b) IC $_{50}$ value of camptothecin on BHK-21 cells is 50 ng/mL.

RESULTS AND DISCUSSION

The dual expression plasmid bearing Bcl-xL anti-apoptotic protein was expected to prolong the cell survival rate and protect cells from apoptosis. To test this hypothesis, apoptosis was induced by doxorubicin and camptothecin in which the anti-apoptotic efficacies are inhibited through over expressing of Bcl-xL (Fiebig et al. 2006; Park et al. 2007; Brunelle and Zhang, 2010). To determine the

concentration of this agents causing 50% cell viability inhibition on BHK-21 cell line, MTT assay was performed. IC_{50} values for doxorubicin and camptothecin were found as 1 µg/mL and 50 ng/mL respectively (Figure 2a and Figure 2b). Apoptosis was induced in BHK-21 cells transfected with pIRES2EGFP, empty vector and pIRES2EGFP-Bcl-xL plasmid with the IC_{50} values of doxorubicin and camptothecin as well as serum deprivation for 48 hrs. Transfected cell ratio expressing EGFP was determined by flow cytometry 48 hrs after apoptosis induction (Figure 3). In pIRES2EGFP/Bcl-xL transfected cells, Bcl-xL is expressed as a fusion protein with EGFP thus EGFP expressing cell ratio equals Bcl-xL expressing cell ratio. In pIRES2EGFP/Bcl-xL transfected cells, transfected cell ratio was significantly higher compared to that of empty vector transfected cells (P < 0.001). Apoptotic cell ratios in EGFP expressing cells were determined by flow cytometry 48 hrs after apoptosis induction (Figure 4). In pIRES2EGFP/Bcl-xL transfected cells, apoptotic cell ratio was significantly lower compared to that of empty vector transfected cells (P < 0.001).

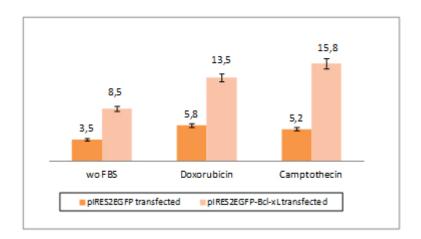


Fig. 3 48 hrs after treatment with apoptosis inducing agents and serum deprivation EGFP expressing cell ratio.

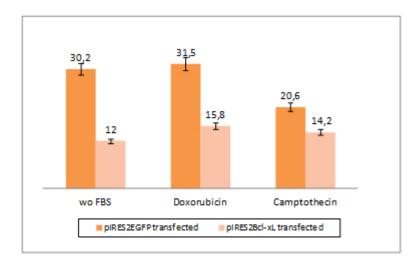


Fig. 4 48 hrs after treatment with apoptosis inducing agents and serum deprivation Casp3⁺ apoptotic cell ratio.

DOI: 10.2225/vol15-issue5-fulltext-2

Bcl-xL over expression is widely used in biotechnological applications including biopharmaceutical protein production, DNA vaccination approaches and therapeutic applications of several diseases (Al-Rubeai and Singh, 1998; Blömer et al. 1998; Kim and Lee, 2000; Huang et al. 2003; Huang et al. 2005; Kim et al. 2005; Yang et al. 2005; Kim et al. 2009; Han et al. 2011; Kim et al. 2011;). Bcl-xL has some advantages like having low molecular weight and more efficient compared to other anti-apoptotic proteins like Bcl-2 (Fiebig et al. 2006).

Bcl-xL is an anti-apoptotic protein that acts by preventing the release of mitochondrial Cytochrome C into cytosol induced by cytotoxic stimuli (Park et al. 2007). Bcl-xL over expression affects the proteins related with cell survival, cell proliferation, and repair of DNA damage thus Bcl-xL over expression affect cell growth in various ways other than apoptosis inhibition (Baik and Lee, 2009).

The growing biotechnological industry has new expectations. Engineering stable cell lines take long period of time in biopharmaceutical protein production thus transient gene expression gains importance. Bcl-xL over expression is one of the promising approaches to increase the recombinant protein expression yields through transient gene expression (Majors et al. 2008). In this study, the efficacy of Bcl-xL anti-apoptotic protein was shown by cell viability assay which are expressing EGFP fused with Bcl-xL. EGFP expressing cell ratio was 2.4 fold higher in pIRES2EGFP/Bcl-xL transfected cells compared to that of pIRES2EGFP transfected cells after serum deprivation; in doxorubicin and camptothecin exposure cells transfected with pIRES2EGFP/Bcl-xL plasmid EGFP expressing cell ratios were 2.3 and 3.0 fold higher compared to pIRES2EGFP transfected cells (Figure 3). Casp3 expressing cell ratio in EGFP transfected cells was 2.5 fold lower in pIRES2EGFP/Bcl-xL transfected cells compared to pIRES2EGFP transfected cells after serum deprivation; in doxorubicin and camptothecin exposure cells transfected with pIRES2EGFP/Bcl-xL plasmid Casp3 expressing cell ratios were 2 and 1.5 fold lower compared to pIRES2EGFP transfected cells (Figure 4). In addition, apoptosis ratio was lower in the cells transfected with Bcl-xL anti-apoptotic protein expressing DNA vaccines compared to control vector transfected cells (Blömer et al. 1998; Kim et al. 2004; Yang et al. 2005). In HEK-293 cells apoptosis ratio was 1.5 fold lower (Kim et al. 2004); in Jurkat T cells 1.7 fold lower (Yang et al. 2005); in PC12 cells 1.48 fold lower (Blömer et al. 1998). Bcl-xL over expression increases antigen specific CD8⁺ T cell responses in human papillomavirus vaccine model (Kim et al. 2004; Huang et al. 2007).

Doxorubicin has been selected as apoptosis inducing agent because it is used as a cancer therapeutic in breast cancers and Bcl-xL over expression inhibits the efficacy of doxorubicin induced apoptosis (Fiebig et al. 2006; Park et al. 2007; Brunelle and Zhang, 2010). Therefore, in this study, in order to determine the anti-apoptotic efficacy of Bcl-xL over expression, apoptosis was induced by doxorubicin. Bcl-xL over expression inhibited apoptosis in MCF-7 breast cancer cell line (Fiebig et al. 2006). Hirsch et al. (1998), reported that the IC_{50} value of doxorubicin was $1\mu g/mL$ as in the case of this study.

The results suggest a transitory role for Bcl-xL over expression in protecting the cellular compartments from DOX invasion and in maintaining the number of mitochondria (Mhawi, 2009). Camptothecin efficacy was also inhibited by Bcl-2 and Bcl-xL over expression (El Assaad et al. 1998; Qian et al. 2009). After 48 hrs treatment with 10 µM camptothecin in MCF-7 cell line over expressing Bcl-xL, apoptosis was inhibited 3.42 fold compared to control MCF-7 (El Assaad et al. 1998). Another study showed that dexamethasone induced Bcl-xL anti-apoptotic pathway and inhibited apoptosis in solid tumor therapies conducted with camptothecin (Qian et al. 2009).

In conclusion, it was shown that *in vitro* transient expression of Bcl-xL efficiently inhibited apoptosis induced by serum deprivation, doxorubicin and camptothecin. Thus, the dual expression plasmid bearing Bcl-xL anti-apoptotic protein could be a good candidate for recombinant protein production and DNA vaccination applications to alter target cell apoptosis for higher recombinant protein yields and prolonged immune responses.

ACKNOWLEDGMENTS

Authors would like to thank Dr Fernando Rodriguez for his invaluable help in the construction of the plasmid and Prof Murat Elibol for critical reading of the manuscript.

Financial support: This work was partly funded by Scientific and Technological Research Council of Turkey (TUBITAK) by grant number 110O809 and Ege University, Science and Technology Center (2011BIL020).

REFERENCES

- AKIYAMA, M.; YAMADA, O.; KANDA, N.; AKITA, S.; KAWANO, T.; OHNO, T.; MIZOGUCHI, H.: ETO. Y.: ANDERSON, K.C. and YAMADA, H. (2002). Telomerase overexpression in K562 leukemia cells protects against apoptosis by serum deprivation and double-stranded DNA break inducing agents, but not against DNA synthesis inhibitors. Cancer Letters, vol. 178, no. 2, p. 187-197. [CrossRef]
- AL-RUBEAI, M. and SINGH, R.P. (1998). Apoptosis in cell culture. Current Opinion in Biotechnology, vol. 9, no. 2, p. 152-156. [CrossRef]
- ANDERSEN, D.C. and KRUMMEN, L. (2002). Recombinant protein expression for therapeutic applications. Current Opinion in Biotechnology, vol. 13, no. 2, p. 117-123. [CrossRef]
- BAIK, J.Y. and LEE, G.M. (2009). A DIGE approach for the assessment of differential expression of the CHO proteome under sodium butyrate addition: Effect of Bcl-xl overexpression. Biotechnology and Bioengineering, vol. 105, no. 2, p. 358-367. [CrossRef
- BLÖMER, U.; KAFRI, T.; RANDOLPH-MOORE, L.; VERMA, I.M. and GAGE, F.H. (1998). Bcl-xL protects adult septal cholinergic neurons from axotomized cell death. Proceedings of the National Academy of Sciences of the United States of America, Neurobiology, vol. 95, no. 5, p. 2603-2608. [CrossRef]
- BRUNELLE, J.K. and ZHANG, B. (2010). Apoptosis assays for quantifying the bioactivity of anticancer drug products. Drug Resistance Updates, vol. 13, no. 6, p. 172-179. [CrossRef]
- EL-ASSAAD, W.; EL-SABBAN, M.; AWARAJI, C.; ABBOUSHI, N. and DBAIBO, G.S. (1998). Distinct sites of action of Bcl-2 and Bcl-x_L in the ceramide pathway of apoptosis. *Biochemical Journal*, vol. 336, no. 3, p. 735-741.
- FIEBIG, A.A.; ZHU, W.; HOLLERBACH, C.; LEBER, B. and ANDREWS, D.W. (2006). Bcl-xL is qualitatively different from and ten times more effective than Bcl-2 when expressed in a breast cancer cell line. BMC Cancer, vol. 6, no. 213. [CrossRef]
- FIGUEROA, B.; SAUERWALD, T.M.; OYLER, G.A.; HARDWICK, J.M. and BETENBAUGH, M.J. (2003). A comparison of the properties of a Bcl-xL variant to the wild-type anti-apoptosis inhibitor in mammalian cell cultures. Metabolic Engineering, vol. 5, no. 4, p. 230-245. [CrossRef]
- FIGUEROA, B.; CHEN, S.; OYLER, G.A.; HARDWICK, M. and BETENBAUGH, M.J. (2004). Aven and Bcl-x_L enhance protection against apoptosis for mammalian cells exposed to various culture conditions. Biotechnology and Bioengineering, vol. 85, no. 6, p. 589-600. [CrossRef]
- HAN, Y.K.; HA, T.K.; KIM, Y.G. and LEE, G.M. (2011). Bcl-x_L overexpression delays the onset of autophagy and apoptosis in hyperosmotic recombinant Chinese hamster ovary cell cultures. Journal of Biotechnology, vol. 156, no. 1, p. 52-55. [CrossRef]
- HIRSCH, T.; DECAUDIN, D.; SUSIN, S.; MARCHETTI, P.; LAROCHETTE, N.; RESCHE-RIGON, M. and KROEMER, G. (1998). PK11195, a ligand of the mitochondrial benzodiazepine receptor, facilitates the induction of apoptosis and reverses Bcl-2-mediated cytoprotection. Experimental Cell Research, vol. 241, no. 2, p. 426-434. [CrossRef]
- HUANG, J.; ITO, Y.; MORIKAWA, M.; UCHIDA, H.; KOBUNE, M.; SASAKI, K.; ABE, T. and HAMADA, H. (2003). Bcl-xL gene transfer protects the heart against ischemia/reperfusion injury. Biochemical and Biophysical Research Communications, vol. 311, no. 1, p. 64-70. [CrossRef]
- HUANG, J.; NAKAMURA, K.; ITO, Y.; UZUKA, T.; MORIKAWA, M.; HIRAI, S.; TOMIHARA, K.; TANAKA, T.; MASUTA, Y.; ISHII, K.; KATO, K. and HAMADA, H. (2005). Bcl-xL gene transfer inhibits bax translocation and prolongs cardiac cold preservation time in rats. Circulation, vol. 112, no. 1, p. 76-83. [CrossRef]
- HUANG, B.; MAO, C.P.; PENG, S.; HE, L.; HUNG, C.F. and WU, T.C. (2007). Intradermal administration of DNA vaccines combining a strategy to bypass antigen processing with a strategy to prolong dendritic cell survival enhances DNA vaccine potency. Vaccine, vol. 25, no. 45, p. 7824-7831. [CrossRef]
- KAUFMANN, S.H. and EARNSHAW, W.C. (2000). Induction of apoptosis by cancer chemotherapy. Experimental Cell Research, vol. 256, no. 1, p. 42-49. [CrossRef]
- KIM, N.S. and LEE, G.M. (2000). Overexpression of bcl-2 inhibits sodium butyrate induced apoptosis in Chinese hamster ovary cells resulting in enhanced humanized antibody production. Biotechnology and Bioengineering, vol. 71, no. 3, p. 184-193. [CrossRef]
- KIM, T.W.; HUNG, C.F.; ZHENG, M.; BOYD, D.A.K.; HE, L.; PAI, S.I. and WU, T.C. (2004). A DNA vaccine coexpressing antigen and an anti-apoptotic molecule further enhances the antigen-specific CD8+ T-cell immune response. Journal of Biomedical Science, vol. 11, no. 4, p. 493-499. [CrossRef]
- KIM, J.H.; CHEN, J.; MAJUMDER, N.; LIN, H.; FALO, L.D. and YOU, Z. (2005). 'Survival gene' Bcl-xl potentiates DNA-raised antitumor immunity. Gene Therapy, vol. 12, no. 20, p. 1517-1525. [CrossRef]
- KIM, Y.G.; KIM, J.Y.; MOHAN, C. and LEE, G.M. (2009). Effect of Bcl-xL overexpression on apoptosis and autophagy in recombinant Chinese hamster ovary cells under nutrient-deprived condition. Biotechnology and Bioengineering, vol. 103, no. 4, p. 757-766. [CrossRef]
- KIM, Y.G.; KIM, J.Y., PARK, B.; AHN, J.O.; JUNG, J.K.; LEE, H.W.; LEE, G.M. and LEE, E.G. (2011). Effect of BclxL overexpression on erythropoietin production in recombinant Chinese hamster ovary cells treated with dimethyl sulfoxide. Process Biochemistry, vol. 46, no. 11, p. 2201-2204. [CrossRef]
- KUTUK, O.; TEMEL, S.G.; TOLUNAY, S. and BASAGA, H. (2010). Aven blocks DNA damage-induced apoptosis by stabilising Bcl-xL. European Journal of Cancer, vol. 46, no. 13, p. 2494-2505. [CrossRef]

- LIEW, J.C.J.; TAN, W.S.; ALITHEEN, N.B.M.; CHAN, E.S. and TEY, B.T. (2010). Over-expression of the X-linked inhibitor of apoptosis protein (XIAP) delays serum deprivation-induced apoptosis in CHO-K1 cells. *Journal of Bioscience and Bioengineering*, vol. 110, no. 3, p. 338-344. [CrossRef]
- LINDENBOIM, L.; DIAMOND, R.; ROTHENBERG, E. and STEIN, R. (1995). Apoptosis induced by serum deprivation of PC12 cells is not preceded by growth arrest and can occur at each phase of the cell cycle. *Cancer Research*, vol. 55, no. 6, p. 1242-1247.
- MAJORS, B.S.; BETENBAUGH, M.J.; PEDERSON, N.E. and CHIANG, G.G. (2008). Enhancement of transient gene expression and culture viability using Chinese hamster ovary cells overexpressing Bcl-xL. *Biotechnology and Bioengineering*, vol. 101, no. 3, p. 567-578. [CrossRef]
- MASTRANGELO, A.J.; HARDWICK, J.M.; BEX, F. and BETENBAUGH, M.J. (2000). Part I. Bcl-2 and bcl-xL limit apoptosis upon infection with alphavirus vectors. *Biotechnology and Bioengineering*, vol. 67, no. 5, p. 544-554. [CrossRef]
- MHAWI, A.A. (2009). Interaction of doxorubicin with the subcellular structures of the sensitive and Bcl-xL-overexpressing MCF-7 cell line: Confocal and low-energy-loss transmission electron microscopy. *Micron*, vol. 40, no. 7, p. 702-712. [CrossRef]
- PARK, S.S.; KIM, M.A.; EOM, Y.W. and CHOI, K.S. (2007). Bcl-xL blocks high dose doxorubicin-induced apoptosis but not low dose doxorubicin-induced cell death through mitotic catastrophe. *Biochemical and Biophysical Research Communications*, vol. 363, no. 4, p. 1044-1049. [CrossRef]
- QIAN, Y.H.; XIAO, Q.; CHEN, H. and XU, J. (2009). Dexamethasone inhibits camptothecin-induced apoptosis in C6-glioma via activation of Stat5/Bcl-xL pathway. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, vol. 1793, no. 5, p. 764-771. [CrossRef]
- THAI, K.; CHOI, J.; FRANZIN, C.M. and MARASSI, F.M. (2005). Bcl-XL as a fusion protein for the high-level expression of membrane-associated proteins. *Protein Science*, vol. 14, no. 4, p. 948-955. [CrossRef]
- YANG, Y.; XIONG, Z.; ZHANG, S.; YAN, Y.; NGUYEN, J.; BERNARD, N.G.; HUIFANG, L.U.; BRENDESE, J.; YANG, F.; WANG, H. and YANG, X.F. (2005). Bcl-xL inhibits T-cell apoptosis induced by expression of SARS coronavirus E protein in the absence of growth factors. *Biochemical Journal*, vol. 392, p. 135-143. [CrossRef]
- ZUSTIAK, M.P.; POLLACK, J.K.; MARTEN, M.R. and BETENBAUGH, M.J. (2008). Feast or famine: Autophagy control and engineering in eukaryotic cell culture. *Current Opinion in Biotechnology*, vol. 19, no. 5, p. 518-526. [CrossRef]

How to reference this article:

GÜLÇE İZ, S.; ÇALIMLIOĞLU, B. and DELILOĞLU, S.İ.G. (2012). Using Bcl-xL anti-apoptotic protein for altering target cell apoptosis. *Electronic Journal of Biotechnology*, vol. 15, no. 5. http://dx.doi.org/10.2225/vol15-issue5-fulltext-2

DOI: 10.2225/vol15-issue5-fulltext-2