Overexpression of Bcl-xl prevents radiation-induced the cell cycle arrest and apoptosis through down regulation of ERK in Hela cell

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1. Introduction

Radiation is one of the most effective DNA damaging reagents. Radiation-induced DNA damage results in the activation of caspase leading to cleavage of cellular protein, leading to cell death. Radio-resistance is known to be associated with anti-apopototic proteins.

Bcl-xl is a well known anti-apoptotic protein and blocks the effects of DNA damaging agents.

2. Methods and Results

We investigated whether Bcl-xl overexpression contributes radiation resistance in Hela cell.

2.1 . Bcl-xl blocked on caspase-specific cleavage of PARP in Hela cells

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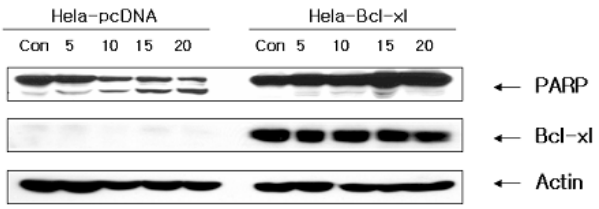


Figure 1. Bcl-xl blocked on caspase-specific cleavage of PARP in Hela cells.

Hela/pcDNA and Hela/Bcl-xl cells were exposed with indicated doses of radiation and incubated for 24hr, and then harvest in lysis buffer. Equal amounts of soluble lysates (30ug) were subjected to electrophoresis.

2.2 Viability of radiation in Hela/pcDNA and Hela/Bcl-xl Cells.

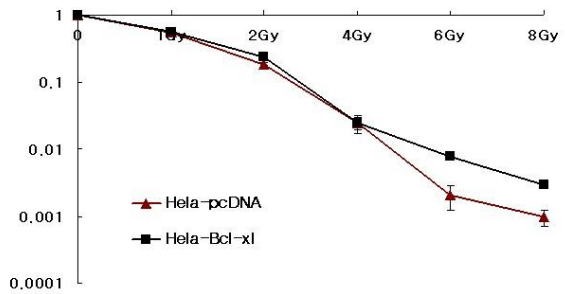
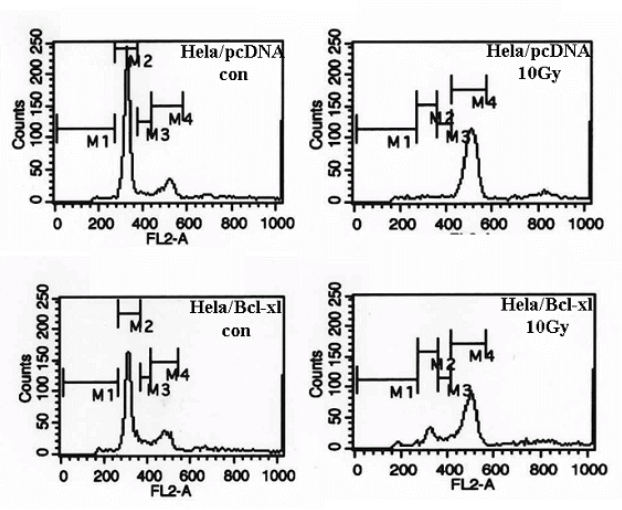


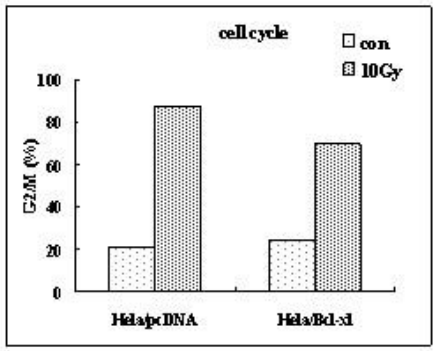
Figure 2. Viability of radiation in Hela/pcDNA and Hela/Bcl-xl Cells.

Effects of radiation on cell viability of Hela/pcDNA cells (open symbols) and Hela/Bcl-xl cells (filled symbols). Hela/pcDNA cells and Hela/Bcl-xl cells were exposed with the indicated dose of radiation and incubated for 15days. Cells were made colony form then stained Trypan-blue. Counts of colony numbers with microscope.

2.3 Bcl-xl shows Resistance against Radiation in Hela cells

A..





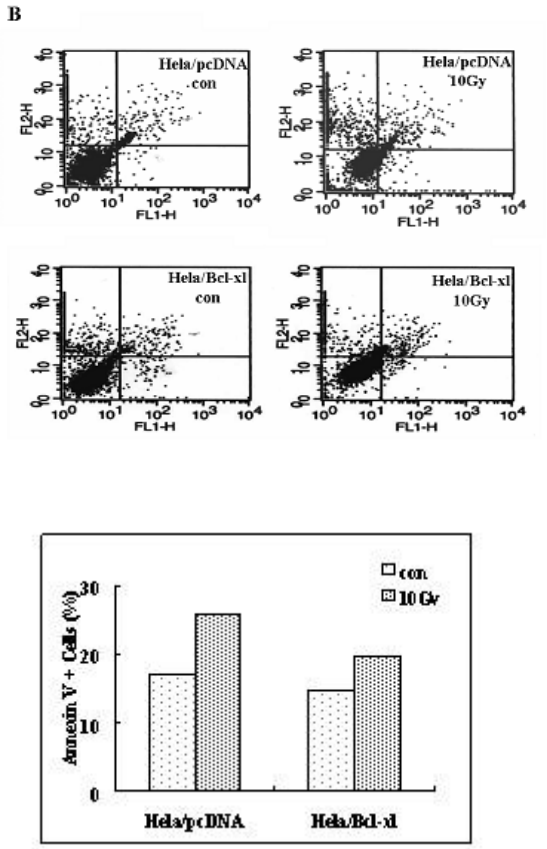


Figure 3. Bcl-xl shows Resistance against Radiation in Hela cells
(A) Facs analysis of G2/M arrest cells. Hela/pcDNA and Hela/Bcl-xl cells were exposed with 10Gy radiation and incubated for 24hr and then evaluated for DNA content after propidium iodide staining. The proportion of G2/M arrest cells is indicated as graph. Data are mean values obtained from three independent experiments and bars represent standard deviations. (B) Hela/pcDNA and Hela/Bcl-xl cells were exposed with 10Gy radiation and incubated for 24hr, after which the percentage of apoptotic cells were determined by Annexin V-FITC/flow cytometry (upper panel).

2.4 Bcl-xl blocks cell death signal with radiation through inhibition of Erk activation.

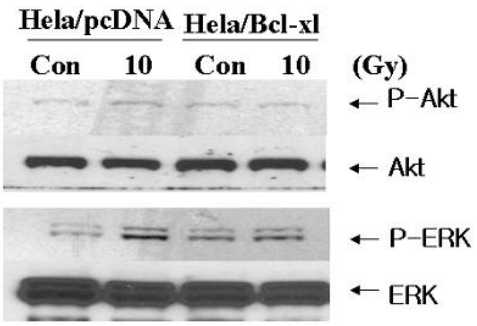


Figure 4. Bcl-xl blocks cell death signal with radiation through inhibition of Erk activation.

Hela/pcDNA and Hela/Bcl-xl cells were exposed with 10Gy radiation then harvest in lysis buffer. Equal amounts of soluble lysates (30mg) were subjected to electrophoresis. The blots were analyzed using a specific antibody against P-ERK, ERK and P-AKT, AKT.

3. Conclusions

Overexpression of Bcl-xl resulted in arrest of G2/M cell cycle. Expression levels of apoptosis-related proteins were activated by radiation. Radiation induced apoptosis through activation of caspase-3 and PARP cleavage in most cancer cells. Bcl-xl overexpression prevented activation of caspase-3 and PARP cleavage in Hela cells. Also, phosporylation of the ERKs was enhanced by radiation in Hela cell.

Our Study showed Bcl-xl overexpression prevents radiation-induced apoptosis through down regulation of ERK and blocks activation of caspases in Hela cell, and suggests that radiation resistance can be overcome by the activation of ERK in Hela cell.

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