Role of Bcl-x_L in Ionizing Radiation-Induced Invasion of Cancer Cells

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1. Introduction

Although radiation therapy is a major therapeutic modality for cancer treatment, previous reports have suggested that ionizing radiation (IR) can promote the invasive and metastatic potential of cancer cells [1]. Successful invasion of cancer cells requires their resistance to apoptosis once the cell has detached from the primary tumor tissue. Bcl-2 family of genes plays a key role in the regulation of apoptotic cell death [2]. Recent studies have revealed that overexpression of Bcl-2, Bcl-w, and Bcl-x_L were associated with tumor progression [2-4]. This study investigated whether Bcl-x_L can mediate the IR-induced invasion of lung cancer cells.

2. Methods and Results

2.1 Cell culture

A549 human lung adenocarcinoma cells were obtained from American Type Culture Collection and grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and gentamicin (50 μg/ml) at 37℃ in a humidified atmosphere containing 5% CO_2.

2.2 Irradiation

Cells were plated in 60-mm dishes and incubated at 37℃ under humidified 5% CO_2 and 95% air in culture medium until 70~80% confluent. Cells were then exposed to γ-rays from a ^{137}Cs γ-ray source [Atomic Energy of Canada] at a dose rate of 3.06 Gy/min.

2.3 Invasion and migration assays

Invasion of A549 cell was measured by the invasion of cells through Matrigel-coated transwell inserts (Corning Inc., Corning, NY). Briefly, transwell inserts with 8-μm pore size were coated with Matrigel (1 mg/ml). Cells were trypsinized, and 200 μl of cell suspension (2×10^5 cells/ml) were added to the Matrigel-coated chamber; the lower compartment was filled with RPMI 1640 conditioned media (1 ml). After incubation for 16~20 h at 37℃, cells on the surface of Matrigel-coated polycarbonate membrane (non-invading cells) were removed with a cotton swab and the migrating cells remaining on the bottom part of the filters were fixed and stained with Diff-Quick kit (Fisher Scientific, Pittsburgh, PA) and then counted under a microscope. Migration assays were done using the same procedure but with uncoated polycarbonate filters.

2.4 Western blot

Cells were lysed in ice-cold lysis buffer containing 1% Triton X-100 in 20 mM Tris-HCl (pH 7.5) and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Lysates were then centrifuged at 14,000 rpm for 20 minutes, equal amounts of proteins (30~50 μg) were separated by 12% SDS-PAGE. The proteins were then electrotransferred to immobilon membranes (Millipore, Bedford, MA), which were subsequently blotted using the indicated antibodies and visualized by chemiluminescence (ECL; Amersham, Arlington Heights, IL).

3. Results

3.1 IR and Bcl-x_L promote the invasiveness of lung cancer cells

IR increased invasive potential of lung cancer cells in a dose-dependent manner and the highest invasiveness observed at 10 Gy of γ-rays (Fig. 1A). Bcl-x_L expression was increased in time dependent manner by exposure of IR (Fig. 1B). To determine whether Bcl-x_L influences the invasiveness of lung cancer cells, Bcl-x_L was overexpressed in A549 cells. As expect, overexpression of Bcl-x_L also promoted their invasion but did not show synergy effect (Fig. 1C). Taken together, IR and Bcl-xL appears to promote the invasion of A549 cells by same mechanism.

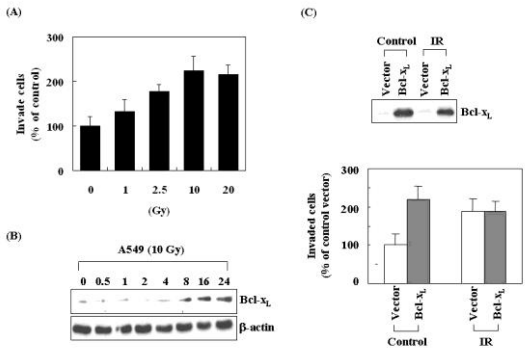

Fig. 1. IR and Bcl-x_L promote the invasive potential of A549 cells. A549 cells were irradiated with 10 Gy of γ-rays. At the end of 24 hr of incubation, Invasion assays (A), and Western blot (B) were performed. Bcl-x_L overexpression was introduced into A549, and then irradiated with or without 10 Gy of γ-rays. Invasion assays (C) were performed.

3.2 MAPK is involved in IR-induced invasion

To investigate whether MAPK acts in IR-induced signaling pathways, we performed Western blot. As indicated in Fig. 2A, IR significantly enhanced the phosphorylation of Akt and p38 and MMP-2 secretion. To further confirm the signaling molecules involved in the IR-induced responses, the cells were irradiated with 10 Gy of γ-rays in the presence or absence of LY294002, SB202190, PD98059, and SP600125 which inhibit PI3K, p38 MAPK, ERK, and JNK, respectively. IR-induced cell invasion was effectively attenuated by the addition of LY294002 (Fig. 2B).

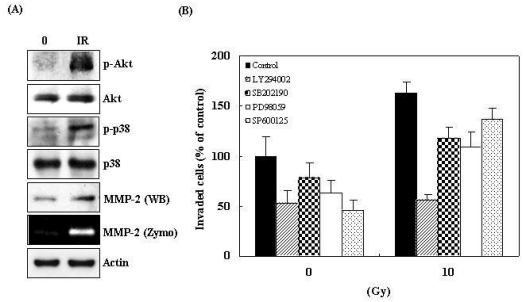

Fig. 2. MAPK is involved in IR-induced invasion. A549 cells were irradiated with 10 Gy of γ-rays. At the end of 24 hr of incubation, Western blot was performed (A), A549 Cells were pretreated with PI3K inhibitor (LY294002, 5 μM), p38 inhibitor (SB202190, 5 μM), Erk inhibitor (PD98059, 5 μM), JNK inhibitor (SP600125, 5 μM) for 30 min, and then irradiated with 10 Gy of γ-rays. At the end of 24 hr of incubation, Invasion assays were performed (B).

3.3 MAPK is involved in Bcl-x_L induced invasion

As shown in Fig. 3A, Bcl-x_L overexpression increased phosphorylation of Akt and p38 and MMP-2 secretion. Moreover, Bcl-x_L failed to increase cell invasion when A549 cells were treated with LY294002 (Fig. 3B). These results suggest that Bcl-x_L may mediate IR-induced invasion of A549 through the up-regulated PI3K/Akt pathway.

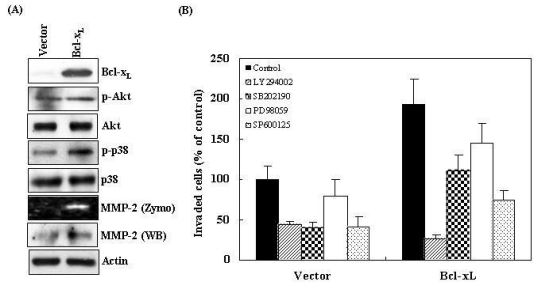

Fig. 3. MAPK is involved in Bcl-x_L-induced invasion. (A) A549 cells were irradiated with 10 Gy of γ-rays. At the end of 24 hr of incubation, Western blot was performed (A), Cells were pretreated with PI3K inhibitor (LY294002, 5 μM), p38 inhibitor (SB202190, 5 μM), Erk inhibitor (PD98059, 5 μM), JNK inhibitor (SP600125, 5 μM) for 30 min, and then irradiated with 10 Gy of γ-rays. At the end of 24 hr of incubation, Invasion assays were performed (B).

3.4 Bcl-x_L is necessary for IR-induced invasion

To determine whether Bcl-x_L expression was crucial to the invasion, the levels of Bcl-x_L were reduced by RNA interference. As shown in Fig. 4A, reduced Bcl-x_L expression inhibited the IR-induced invasion compared with nonsilencing control cells. Moreover, IR-induced phosphorylation of Akt and p38 and MMP-2 secretion were reduced when the cells were treated with Bcl-x_L siRNA (Fig. 4B).

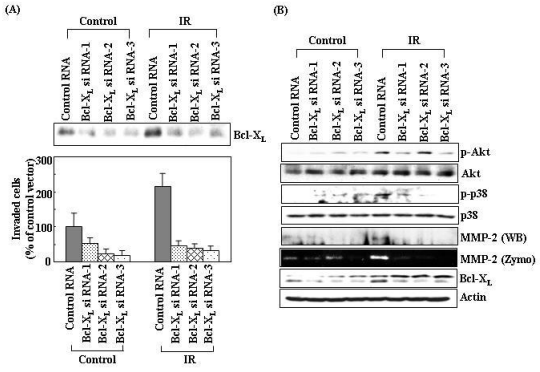

Fig. 4. Bcl-x_L mediates IR-induced invasion. Bcl-x_L siRNA was introduced into A549, and then irradiated with 10 Gy of γ-rays. After 24 hr of incubation, Invasion assay (A) and, Western blot (B) were performed.

3. Conclusions

In the present study, we showed that Bcl-x_L is a critical mediator of IR-induced invasion through the up-regulated phosphorylation of Akt and p38 pathway and MMP-2 secretion.

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