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Bcl-X_L and STAT3 mediate malignant actions of γ-irradiation in lung cancer cells

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Previous reports suggest that, in addition to its therapeutic effects, ionizing radiation (IR) increases the invasiveness of surviving cancer cells. Here, we demonstrate that this activity of IR in lung cancer cells is mediated by a signaling pathway involving p38 kinase, phosphoinositide 3-kinase, Akt, and matrix metalloproteinase (MMP-2). The invasion-promoting doses of IR also increased and reduced the levels of vimentin and E-cadherin, respectively, both of which are markers for the epithelial–mesenchymal transition (EMT). Interestingly, all of these malignant actions of IR were mimicked by the overexpression of Bcl-X_L, a pro-survival member of the Bcl-2 family, in lung cancer cells. Moreover, both RNA and protein levels of Bcl-X_L were elevated upon irradiation of the cells, and the prevention of this event using small-interfering RNAs of Bcl-X_L reduced the ability of IR to promote invasion signals and EMT-associated events. This suggests that Bcl-X_L functions as a signaling mediator of the malignant effects of IR. It was also demonstrated that IR enhances signal transducer and activator of transcription 3 (STAT3) phosphorylation, and the reduction of STAT3 levels via RNA interference prevented IR-induced Bcl-X_L accumulation, and thus all the tested Bcl-X_L-dependent events. Overall, the data suggest that IR induces Bcl-X_L accumulation via STAT3, which then promotes cancer cell invasion and EMT-associated markers. Our findings demonstrate a novel function of Bcl-X_L in cancer, and also advance our understanding of the malignant actions of IR significantly. (*Cancer Sci* 2010; 101: 1417–1423)

Although ionizing radiation (IR) is widely utilized for cancer therapy,[1–3] recent studies have shown that IR also exerts undesirable effects on cancer cells. For example, the irradiation of various types of cancer cells, including glioma,[4] hepatocellular carcinoma,[5] pancreatic cancer,[6] and meningioma cells,[7] with sublethal doses of IR resulted in an increase in their invasiveness in culture. Moreover, a large number of studies using animal models have demonstrated that local radiotherapy administered to primary tumors speeds their metastatic growth *in vivo*,[8–10] thereby suggesting that besides its therapeutic effects, IR promotes the malignant behaviors of surviving cancer cells. This latter effect of IR is also consistent with the finding that local failure after radiotherapy increases the probability that distant metastasis will develop in cancer patients.[11] Therefore, preventing such undesirable action of IR may be an essential component of efforts to improve the efficacy of radiotherapy.

In order to develop a strategy toward this objective, the mechanism by which IR promotes cancer cell invasion should be delineated. Previous studies using glioma cells have suggested that IR stimulates signaling components, such as p38 kinase, phosphoinositide 3-kinase (PI3K), and Akt, leading to matrix metalloproteinase-2 (MMP-2) expression and thus enhanced cell invasion.[4] The ability of IR to induce the PI3K/Akt-dependent invasion pathway was also proposed using hepatocellular carcinoma cells.[5] However, apart from these reports, limited information is currently available regarding the cellular components involved in the invasion-promoting action of IR.

The Bcl-2 family proteins are key regulators of cellular viability.[12–15] While certain members of the family, such as Bax and Bak, promote apoptosis, other members, such as Bcl-2, Bcl-X_L, and Bcl-w bind directly to the pro-apoptotic members to block their apoptotic actions, thus bolstering cell survival. Interestingly, evidence has been accumulating recently that suggests additional functions of the pro-survival members in cancer. First, the overexpression of Bcl-2,[16–18] Bcl-X_L,[19–21] or Bcl-w[22,23] in the specified cancer cells resulted in increases in the migratory and invasive potential of the cells. These effects of Bcl-2[16,17] and Bcl-w[22,23] appear to be the result of their ability to stimulate invasion-promoting signaling events. It has also been demonstrated that the overexpression of Bcl-2[18] or Bcl-X_L[19,21] promotes the metastatic growth of cancer cells in an animal model. Therefore, it appears that the role of pro-survival Bcl-2 family members in cancer is not confined simply to the survival of cancer cells, but that they also support the invasion and metastasis of cancer cells. Importantly, this latter newly proposed function of pro-survival members appears clinically relevant, considering that analyses of patient samples have revealed that Bcl-w is expressed in gastric cancer[24] and glioma multiforme,[25] particularly in cancer cells evidencing infiltrative morphology. It was similarly reported that Bcl-X_L expression in cases of colon cancer was associated with lymph-node metastasis of the cancer cells.[26]

Interestingly, certain, if not all, pro-survival Bcl-2 family members respond to IR. For example, cellular levels of Bcl-2[27] and Bcl-X_L[28] were shown to be elevated upon exposure of various cancer cell types to IR. Although the mechanism underlying this phenomenon remains largely unknown, the increase in Bcl-2 and Bcl-X_L levels may contribute to the resistance of cancer cells to radiotherapy.[29,30] In this regard, it might be speculated that IR-induced pro-survival members also mediate the ability of IR to promote cancer cell invasion. The current study was undertaken in order to investigate this possibility directly. In service of this purpose, we focused on Bcl-X_L as a model. Human lung cancer cells were used, given the frequent application of radiotherapy to lung cancer patients.[31]

Materials and Methods

Antibodies. Antibodies were purchased from the following institutions: anti-Bcl-X_L, -Bcl-2, -Bak, and -Akt from Santa Cruz Biotechnology (Santa Cruz, CA, USA), anti-phospho-Akt, -p38 kinase, -phospho-p38 kinase, -signal transducer and activator of transcription 3 (STAT3), and -phospho-STAT3 from Cell Signaling (Beverly, MA, USA), anti-E-cadherin and -vimentin

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from BD Pharmingen (San Diego, CA, USA), anti-MMP-2 from Calbiochem (La Jolla, CA, USA), Alexa Fluor-488 (green) and Alexa Fluor-555 (red) secondary antibodies from Molecular Probes (Carlsbad, CA, USA).

Cell culture, transfection, and treatment. Human A549 and H460 lung cancer cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and gentamicin (50 μg/mL). The expression constructs for Bcl-X_L were prepared using pcDNA3 vectors. These expression vectors, as well as small-interfering RNAs (siRNAs) of Bcl-X_L and STAT3 (Ambion, Austin, TX, USA) were introduced into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). To irradiate the cells/transfectants, they (3×10^5) were plated on 60 mm dishes, incubated until 70–80% confluence was reached, and then exposed to γ-rays from a ^{137}Cs γ-ray source (Atomic Energy of Canada, Mississauga, ON, Canada) at a dose rate of 3 Gy/min. Where specified, cells were administered pharmacological inhibitors (Calbiochem) 30 min before irradiation. All of the treatments employed herein did not significantly influence cellular viability, as determined by flow cytometry[24] or Trypan blue exclusion assays.

Invasion assay. Cells were seeded onto the upper surfaces of Matrigel-coated polycarbonate filters (BD Biosciences, Bedford, MA, USA) in modified Boyden chambers (Corning, Corning, NY, USA), and analyzed for their invasiveness as described previously.[22]

Western blot analysis. The cell lysates were prepared via a previously reported method.[32] To compare levels of secreted MMP-2, conditioned media were prepared via 24 h of incubation of the cells in serum-free medium. Proteins in the lysates or conditioned media were separated by SDS–PAGE, electrotransferred to Immobilon membranes (Millipore, Bedford, MA, USA), subsequently blotted using the specified antibodies, and visualized with the ECL detection system (Amersham, Uppsala, Sweden).

Immunofluorescence microscopy. Cells were grown on glass coverslips for 24 h. Where indicated, the cells were irradiated, and subsequently incubated for an additional 24 h. The cells were then fixed in 2% paraformaldehyde, permeabilized in PBS containing 0.2% Triton X-100, blocked for 15 min with 1% normal goat serum in PBS at room temperature, and incubated for 16 h at 4°C with anti-vimentin or -E-cadherin. The cells were washed and incubated for 1 h at room temperature with Alexa Fluor-488 (green) and Alexa Fluor-568 (red) secondary antibodies, which were used for vimentin and E-cadherin, respectively. The coverslips were again washed, mounted on slides with Fluorescence Mounting Medium (Dako Diagnostics, Mississauga, ON, Canada), and analyzed via confocal laser scanning microscopy (Zeiss LSM 710; Carl Zeiss, Oberkochen, Germany).[33]

Reverse transcription–polymerase chain reaction (RT-PCR). Total RNA was extracted using Trizol reagent in accordance with the manufacturer's instructions (Invitrogen). The RT reactions were performed using total RNA as a template and a StarScript RT kit (Stratagene, La Jolla, CA, USA). PCR amplification was conducted using the following primers: Bcl-X_L, 5′-ATCAATGGCAACCCATCCTG-3′ and 5′-TTGTCTACGCTTTCCACGCA-3′; GAPDH, 5′-CATCTCTGCCCCCTCTGCTGA-3′ and 5′-GGATGACCTTGCCCACAGCCT-3′. PCR products were subjected to electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed.[34]

Statistical analysis. Results were analyzed for statistical significance via the Student's *t*-test. Differences were considered significant at $P < 0.05$.

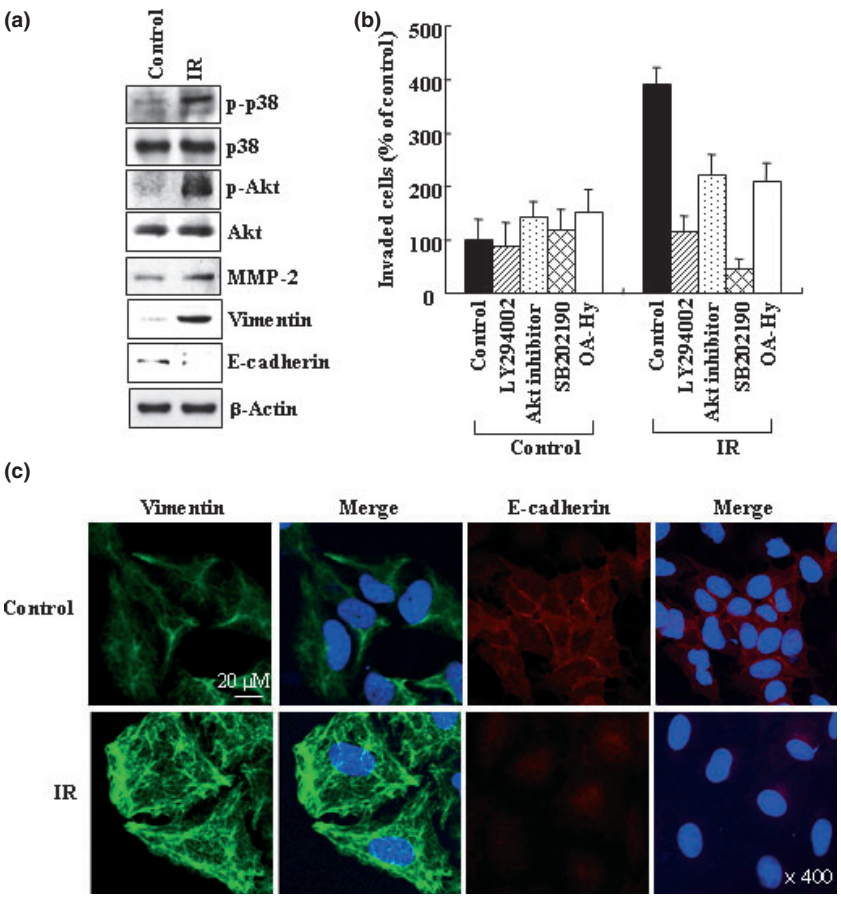

Fig. 1. Ionizing radiation (IR) promotes malignant behaviors in A549 cells. (a) A549 cells were irradiated with 10 Gy of γ-rays. After 24 h of incubation, cell lysates and conditioned media were prepared. Cell lysates were analyzed for p-p38, p38, p-Akt, Akt, vimentin, and E-cadherin levels by western blotting using β-actin as a loading control. The conditioned media were analyzed to compare the levels of secreted matrix metalloproteinase (MMP-2). (b) A549 cells were incubated with or without inhibitors for phosphoinositide 3-kinase (PI3K) (LY294002, 5 μM), Akt (Akt inhibitor I, 5 μM), p38 kinase (SB202190, 5 μM), or MMP-2 (OA-Hy, 5 μM) for 30 min, and then irradiated. After 24 h of incubation, cellular invasiveness was compared on Matrigel-coated polycarbonate filters. The experiment was repeated three times, and the means and SDs were determined. (c) The control and irradiated cells were immunostained to analyze vimentin and E-cadherin. DAPI staining to detect nuclei was also carried out. Stained cells were visualized with a confocal microscope.

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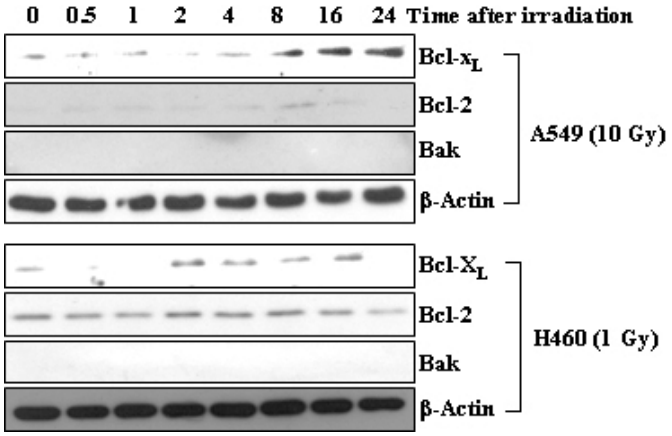

Fig. 2. Bcl-X_L is a common target of ionizing radiation (IR) in both A549 and H460 cells. A549 and H460 cells were exposed to sublethal doses of IR, 10 and 1 Gy, respectively. At the indicated times after irradiation, the levels of Bcl-X_L, Bcl-2, and Bak were compared via western blotting.

Results

Ionizing radiation promotes malignant behaviors of A549 cells. It was previously reported that IR promoted glioma cell invasion by inducing MMP-2 expression via p38 kinase, PI3K, and Akt.[4] To confirm this action of IR in lung cancer cells, A549 cells were irradiated with sublethal doses of γ-rays (10 Gy). This treatment induced an increase in the invasiveness of the cells, which was apparent at 16–24 h after irradiation, but not at 8 h or earlier (see below). Therefore, unless otherwise specified, 24 h was selected for further analyses of the irradiated cells. Western blot analysis showed that the irradiation also resulted in an increase in the phosphoryation levels of p38 kinase and Akt, as well as the protein levels of MMP-2 (Fig. 1a). Moreover, the invasion-promoting effect of IR was attenuated when the cells were irradiated in the presence of inhibitors for p38 kinase (SB202190), PI3K (LY294002), Akt (Akt inhibitor), or MMP-2 (OA-Hy). This confirms the involvement of these components in the IR-induced invasion of A549 cells. Given that cancer cells' invasiveness is related closely with their epithelial–mesenchymal transition (EMT),[35,36] immunostaining (Fig. 1c) and western blotting (Fig. 1a) were conducted to analyze vimentin and E-cadherin, markers for mesenchyma and epithelium, respectively. The irradiation indeed induced an increase and decrease in the levels of vimentin and E-cadherin, respectively. This verifies the ability of IR to induce EMT-associated features in A549 cells, as has also been proposed by other researchers.[37] Overall, the data indicate that IR promotes malignant behaviors of lung cancer cells.

Bcl-X_L is a common target of IR in multiple lung cancer cell types. In an effort to determine whether or not a pro-survival Bcl-2 family member is involved in this effect of IR, it was first necessary to determine which member of the family responds to IR in lung cancer cells. Figure 2 shows that levels of both Bcl-X_L and Bcl-2 proteins were increased in the irradiated A549 cells, which became apparent at ~8 h after irradiation and thus before the elevation of cellular invasiveness. Such effects appear to reflect a specific action of IR, as Bak was not detected regardless of irradiation. An increase in

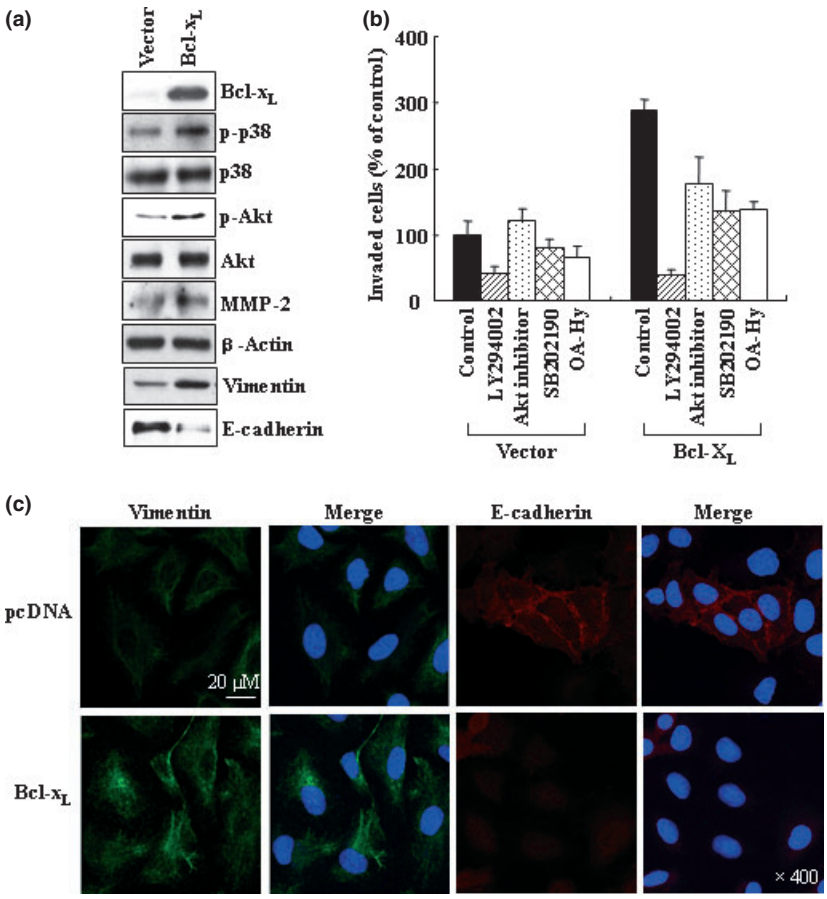

Fig. 3. Bcl-X_L overexpression mimics the effects of ionizing radiation (IR). (a) A549 cells were transiently transfected with empty pcDNA3 vectors or vectors containing Bcl-X_L cDNA. The levels of the indicated components were compared via western blotting. (b) The control and Bcl-X_L transfectants were incubated with or without indicated inhibitors for 30 min, then analyzed for their invasiveness. (c) The transfectants were immunostained and analyzed for vimentin and E-cadherin.

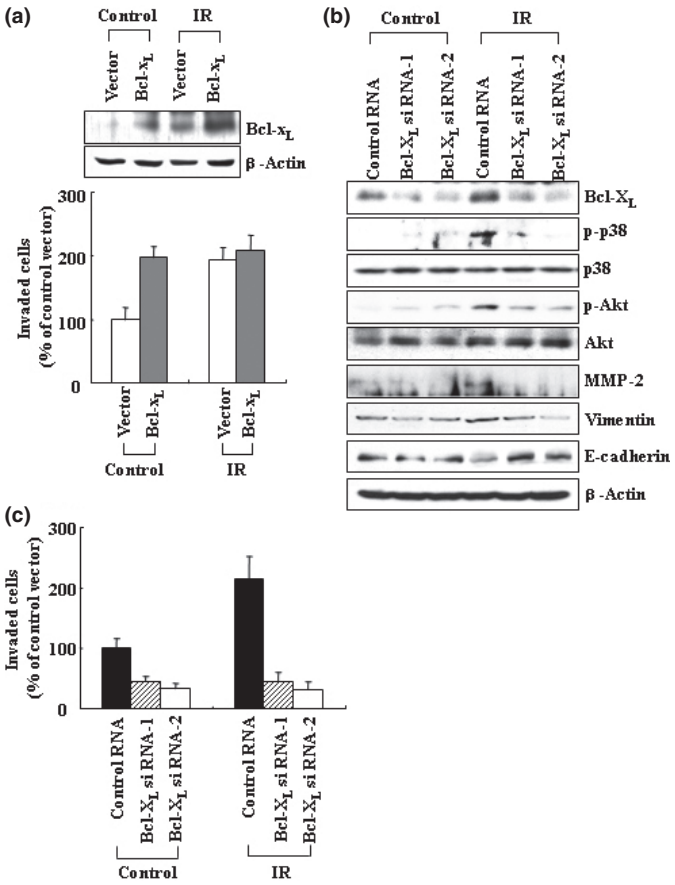

Fig. 4. Bcl-X_L mediates the malignant actions of ionizing radiation (IR). (a) The transfectants were irradiated (10 Gy), and incubated for 24 h. Cellular levels of Bcl-X_L and cellular invasiveness were compared. (b) Control and Bcl-X_L siRNAs were introduced into A549 cells. After 24 h of incubation, the cells were irradiated, then incubated for an additional 24 h. Levels of the indicated components were compared via western blotting. (c) Cellular invasiveness was analyzed.

Bcl-X_L levels was also noted when H460 lung cancer cells were irradiated with sublethal doses of γ-rays (1 Gy). By way of contrast, Bcl-2 levels in H460 cells were not, or were only minimally, elevated by the same treatment. This suggests that Bcl-X_L is a more common responder to IR, at least in these experimental settings. Therefore, Bcl-X_L was selected as a model for further study.

Bcl-X_L mimics IR to promote malignant features of A549 cells. To determine whether Bcl-X_L augments the malignant features of lung cancer cells, Bcl-X_L was overexpressed in A549 cells. This treatment indeed increased the phosphorylation levels of p38 kinase and Akt, protein levels of MMP-2 (Fig. 3a), and the invasiveness of A549 cells (Fig. 3b). This latter event was again shown to be reduced by the use of inhibitors for p38 kinase, PI3K, Akt, and MMP-2. Moreover, the overexpression of Bcl-X_L increased and decreased the levels of vimentin and E-cadherin, respectively (Fig. 3a,c). These effects of Bcl-X_L are very reminiscent of those of IR, thereby indicating that Bcl-X_L mimics the actions of IR to stimulate malignant signals in A549 cells.

Bcl-X_L mediates the malignant actions of IR. We next irradiated the control and Bcl-X_L transfectants with sublethal doses of IR. As compared to single treatment with either Bcl-X_L transfection or irradiation, combination treatment caused a further increase in Bcl-X_L levels, but induced no further increase in cellular invasiveness (Fig. 4a). Therefore, Bcl-X_L levels induced by either its transfection or irradiation might effectively promote cellular invasiveness. Our data support the hypothesis that Bcl-X_L operates in the IR-induced signaling pathway. To more directly confirm this, IR-induced Bcl-X_L accumulation was prevented via RNA interference. This treatment reduced the ability of IR to increase the phosphorylation levels of p38 kinase and Akt, MMP-2 protein levels (Fig. 4b), and thus cellular invasiveness (Fig. 4c). Ionizing radiation also failed to increase the level of vimentin and reduce E-cadherin levels when Bcl-X_L levels were reduced (Fig. 4b). Therefore, IR appears to induce all these malignant signals or events in a Bcl-X_L-dependent manner. Overall, the data suggest that Bcl-X_L functions as a signaling component that mediates the actions of IR to promote cancer cell invasion and EMT.

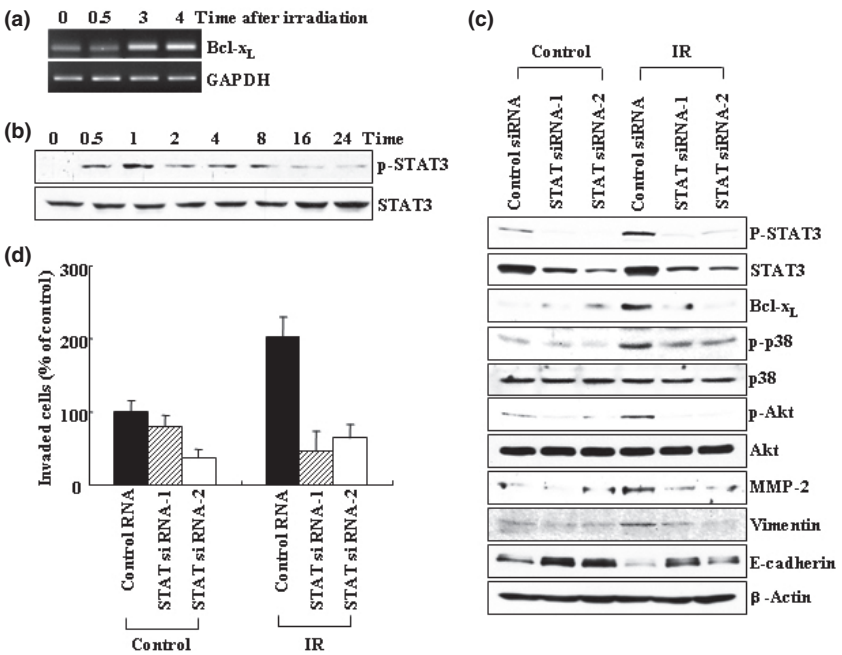

Fig. 5. Signal transducer and activator of transcription 3 (STAT3) mediates ionizing radiation (IR)-induced Bcl-X_L accumulation. (a) A549 cells were irradiated. At the indicated times, the levels of Bcl-X_L mRNA were compared via RT-PCR, using GAPDH as an internal control. (b) The irradiated cells were analyzed for levels of STAT3 protein and its phosphorylation via western blotting. (c) Control and STAT3 siRNAs were introduced into A549 cells. After 24 h of incubation, the cells were irradiated and incubated for an additional 24 h. Levels of the indicated components were compare via western blotting. (d) Cellular invasiveness was compared.

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Signal transducer and activator of transcription 3 (STAT3) mediates IR-induced Bcl-X_L accumulation. We subsequently assessed the cellular factors involved in IR-induced Bcl-X_L accumulation. Reverse transcription–polymerase chain reaction (RT-PCR) showed that the mRNA levels of Bcl-X_L increased in response to IR (Fig. 5a), suggesting that IR induces Bcl-X_L accumulation at the transcriptional level. This view led us to focus on STAT3, a transcription factor which can directly regulate the Bcl-X_L gene.[38,39] Indeed, IR increased the phosphorylation levels of STAT3 without significantly affecting its protein levels (Fig. 5b). This verifies the ability of IR to activate STAT3, which was also proposed under other experimental conditions.[40,41] Importantly, IR-induced STAT3 phosphorylation was observed from 30 min after irradiation, and thus precedes IR-induced Bcl-X_L accumulation. Consistent with this time sequence, the reduction of STAT3 levels by RNA interference attenuated the ability of IR to induce Bcl-X_L accumulation and thus Bcl-X_L-dependent downstream events, including increases in phosphorylation or protein levels of p38 kinase, Akt, MMP-2, and vimentin, as well as reductions in E-cadherin levels (Fig. 5c). Moreover, IR failed to increase cells' invasiveness when STAT3 levels were reduced (Fig. 5d). Overall, the data suggest that STAT3 mediates the ability of IR to induce invasion signals and EMT markers via the induction of Bcl-X_L accumulation.

Transient action of IR. Figure 5(b) also shows that the levels of IR-induced STAT3 phosphorylation were not stable, and decreased with continued incubation. This may reflect a downregulation of IR-induced malignant signals. Indeed, Bcl-X_L, MMP-2, vimentin, and E-cadherin were restored to their original levels when incubation continued for up to 48–72 h (Fig. 6a). At this stage of post-irradiation, the irradiated cells were not superior to untreated control cells in terms of their invasiveness (Fig. 6b). Therefore, the malignant effects of IR appear to be reversible.

Cell-type specificity of IR action. Given that IR induces Bcl-X_L accumulation not only in A549 cells but also in H460 cells (Fig. 2), we attempted to determine whether IR also functions in a Bcl-X_L-dependent manner in the latter cell type. As in the case of A549 cells, the irradiation of H460 cells with doses of IR that elevated Bcl-X_L levels also resulted in an increase in the phosphorylation or protein levels of p38 kinase, Akt, MMP-2, and vimentin, as well as a reduction in E-cadherin levels (Fig. 7a). This confirms the ability of IR to stimulate invasion signals and EMT markers in H460 cells. Importantly, all of the effects of IR were mimicked by Bcl-X_L overexpression (Fig. 7b), and the reduction of Bcl-X_L accumulation by its RNA interference prevented the IR-induced invasion of H460 cells (Fig. 7c), thereby suggesting that Bcl-X_L functions as a mediator of IR action in H460 cells. Moreover, irradiation also increased the levels of STAT3 phosphorylation, and the prevention of this event using STAT3 siRNAs abolished the actions of IR to stimulate or induce Bcl-X_L, p38 kinase, Akt, MMP-2, and vimentin, to reduce E-cadherin levels (Fig. 7d), and to promote the invasion of H460 cells (Fig. 7e). Therefore, STAT3 also appears to mediate IR-induced Bcl-X_L accumulation in H460 cells. The data collectively suggest that Bcl-X_L and STAT3 mediate the ability of IR to induce invasion and EMT markers in multiple lung cancer cell types.

Discussion

We have shown that IR enhances the invasiveness of lung cancer cells, A549 and H460, by stimulating a cellular pathway involving p38 kinase, Akt, and MMP-2. These effects of IR were also accompanied by EMT-associated events, including an increase in vimentin levels and a reduction in E-cadherin levels. Therefore, we subsequently investigated the possible role of a pro-survival Bcl-2 family member in these malignant actions of IR in this system.

Our data do indeed suggest that Bcl-X_L functions as a signaling mediator of the malignant actions of IR. We focused on Bcl-X_L because levels of this molecule in both A549 and H460 cells were shown to be elevated upon irradiation with invasion-promoting doses of IR. Similar results were obtained when the alternative lung cancer cells, H1299 and Calu-1, were analyzed (data not shown), suggesting that Bcl-X_L is a common responder to IR in multiple lung cancer cell types. Moreover, the observation that the invasiveness of lung cancer cells is increased and decreased upon the overexpression and RNA interference (respectively) of Bcl-X_L confirms the ability of Bcl-X_L to promote cell invasion in this system. This function of Bcl-X_L was blocked by the use of inhibitors for p38 kinase, PI3K, Akt, and MMP-2, suggesting that these components are also involved in Bcl-X_L-induced invasion. Therefore, Bcl-X_L and IR appear to promote cell invasion via a commonly shared pathway. This view was supported further by the finding that Bcl-X_L overexpression mimicked the actions of IR in influencing phosphorylation or protein levels of p38 kinase, Akt, MMP-2, vimentin, and E-cadherin. Importantly, the ability of IR to influence all

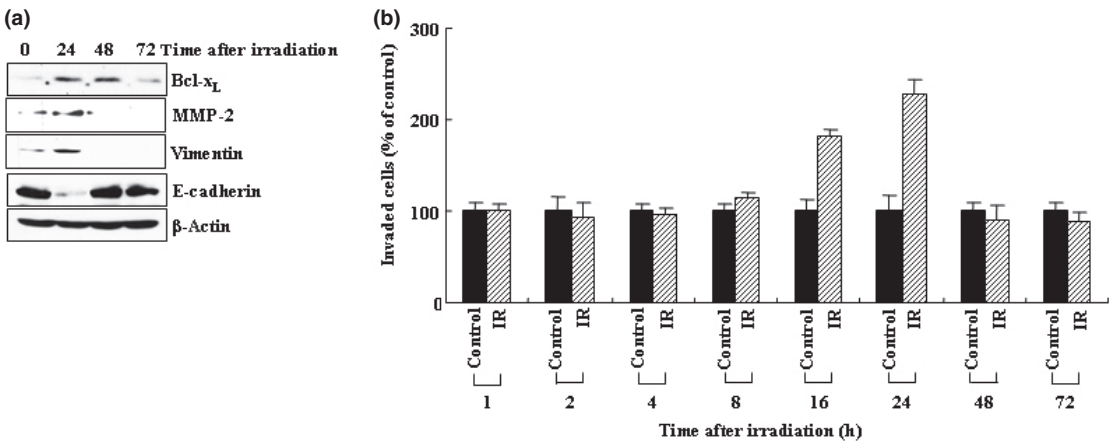

Fig. 6. Ionizing radiation (IR) promotes invasion in a transient mode. (a) Levels of Bcl-X_L, matrix metalloproteinase-2 (MMP-2), vimentin, and E-cadherin were analyzed by western blotting at the indicated times after irradiation. (b) Cellular invasiveness was compared at the times specified.

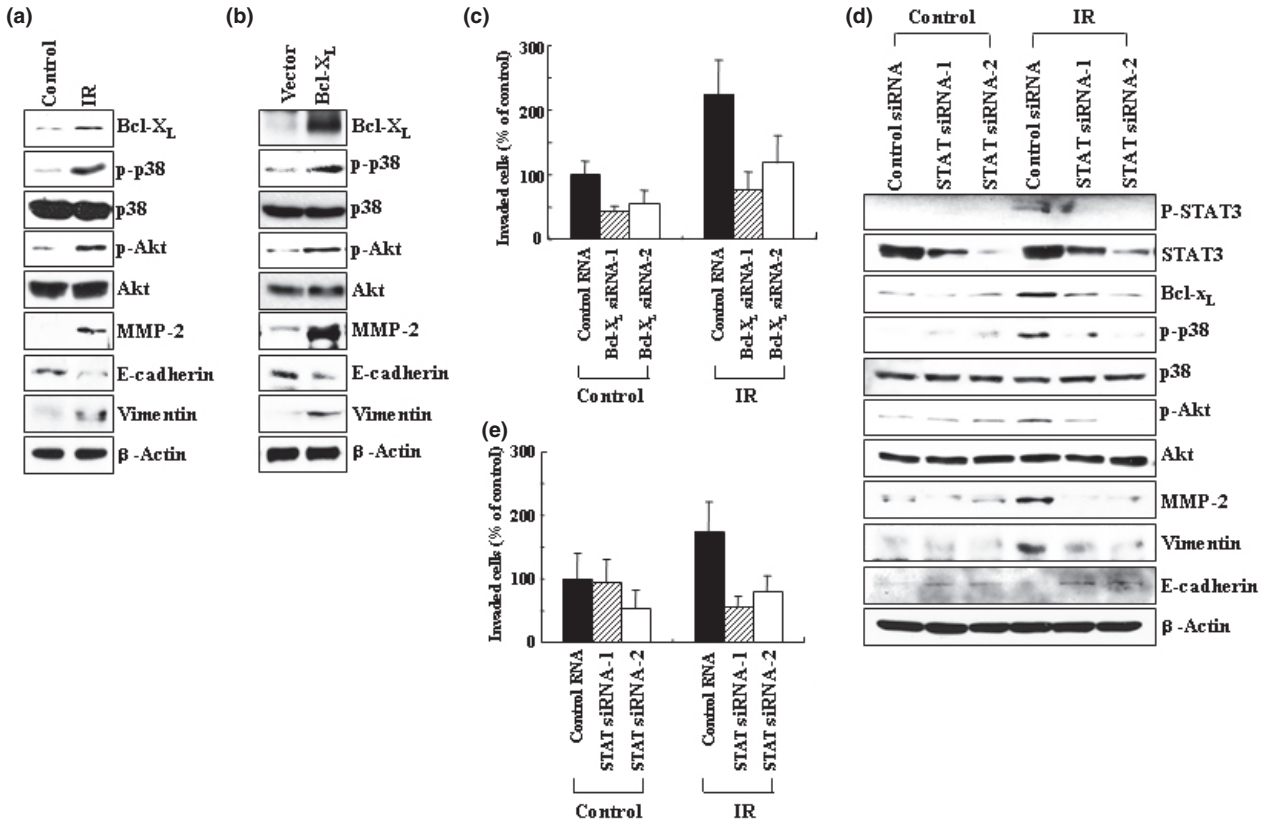

Fig. 7. Role of Bcl-X_L and signal transducer and activator of transcription 3 (STAT3) in H460 cells. (a) H460 cells were irradiated (1 Gy), and incubated for 24 h. Levels of the indicated components were analyzed by western blotting. (b) H460 cells were transiently transfected with the control and Bcl-X_L expression vectors. Levels of the indicated components were compared. (c) Control and Bcl-X_L siRNAs were introduced into H460 cells. After 24 h of incubation, the cells were irradiated, incubated for an additional 24 h, and analyzed for invasiveness. (d) Control and STAT3 siRNAs were introduced into H460 cells. After 24 h of incubation, the cells were irradiated, incubated for an additional 24 h, and subsequently analyzed for the relevant components by western blotting. (e) Cellular invasiveness was compared.

these components and thus to enhance cells' invasiveness was attenuated in cases in which the levels of Bcl-X_L were reduced by RNA interference. Overall, our data indicate that IR promotes cell invasion and EMT-associated events via the induction of Bcl-X_L accumulation. To the best of our knowledge, this is the first report to demonstrate directly that a pro-survival Bcl-2 family member can function as a signaling mediator of an extracellular stimulus. This role of Bcl-X_L can be contrasted with its anti-apoptotic action, in which Bcl-X_L functions as a blocker of the apoptotic signal.[42,43] It has been reported consistently that the ability of Bcl-X_L to suppress apoptosis and to enhance invasiveness can be dissociated in a manner dependent on the time after Bcl-X_L induction.[20]

We have also determined that STAT3 acts upstream of Bcl-X_L in the IR-induced invasion pathway. The involvement of a transcription factor in IR-induced Bcl-X_L accumulation was initially implied by the finding that IR increases not only Bcl-X_L protein levels, but also its mRNA levels. Given that STAT3 can induce Bcl-X_L expression under other experimental conditions,[38,44] we focused on the role of STAT3 in this system. We confirmed that STAT3 responds to IR by showing that IR enhances STAT3 phosphorylation. This event preceded Bcl-X_L accumulation, suggesting that STAT3 is involved in IR-induced Bcl-X_L accumulation. This latter event was indeed abolished when STAT3 levels were reduced as the result of RNA interference. Moreover, our finding that the reduction in STAT3 levels also attenuates the ability of IR to promote all of the Bcl-X_L-dependent downstream events tested and the invasiveness of the cancer cells directly supports the notion that STAT3 mediates the ability of IR to induce invasion and EMT-associated events via the induction of Bcl-X_L accumulation. In addition to these functions in IR-induced invasion, STAT3 and Bcl-X_L may also contribute to the constitutive invasion activities of cancer cells. This latter view is based on the observation that phosphorylated STAT3 and Bcl-X_L exist in untreated control cells, and that the reduction of their levels via RNA interference can attenuate the invasiveness of the cells (Figs 4c,5d). While STAT3 has been implicated in the regulation of the growth and survival of cancer cells,[45] this study suggests its additional functions and illustrates a novel mechanism of its action.

In summary, we have demonstrated that IR induces Bcl-X_L accumulation via STAT3, which then promotes invasion and EMT-associated events in lung cancer cells. This finding significantly advances our understanding not only of the role of Bcl-X_L in cancer, but also of the mechanism underlying the malignant actions of IR.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

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