Activity-related modulations of Bak and Bax critically depend on Src-PKCδ-p38MAPK signalling during radiation-induced apoptotic cell death in non-small cell lung cancer cells

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1. Introduction

Sensitivity of tumor cells to radiation is a critical determinant of the probability of local control and ultimately, of cure. Thus, one approach to improving the outcome of radiation therapy depends on determining which factors lead to tumor cell sensitivity to therapy. It has been shown that many factors affect susceptibility of tumor cells to ionizing radiation. Among them intracellular signaling molecules and apoptotic factors seem to play an important role in determining the intrinsic radiosensitivity of tumor cells.

In this study, we investigate signaling pathways mediating radiation-induced apoptotic cell death that play an important role in determining the sensitivity of non-small cell lung cancer cells to the ionizing radiation. We demonstrate that tyrosine phosphorylation of PKCδ by Src kinase induces mitochondrial activation-dependent apoptotic cell death after ionizing irradiation through p38MAPK-mediated activity-related modulation of pro-apoptotic proteins Bax and Bak in human non-small cell lung cancer cells, and suggest that any attempt at dissecting further the specific signal transduction pathway involved in the initiation of apoptotic cell death will guide the development of novel strategies in targeted-radiation therapy.

2. Methods

Radiation-induced apoptotic cell death was determined by flow cytometric analysis. Involvement of the mitochondrial pathway in radiation-induced cell death was examined by monitoring of the mitochondria membrane potential, cytochrome *c* release, Bax translocation, and Bcl-2 phosphorylation. Subcellular redistributions of apoptosis inducing factor (AIF) were detected using Western blot analysis after subcellular fractionation and confocal microscopic analysis. Phosphorylation of Bcl-2 by JNK after irradiation was determined by immune complex kinase assay.

3. Results and Conclusion

Since it has been shown that the proapoptotic Bcl-2 family members Bax and Bak are crucial to the mitochondrial activation-mediated apoptosis pathways, we investigated whether γ-radiation treatment induces activation of Bax or Bak. We first analyzed activity-related conformational changes in Bax and Bak by flow-cytometric analysis with antibodies recognizing N-terminal epitopes of Bax or Bak. As shown in Fig. 1A, γ-radiation treatment resulted in activity-related modulation of both Bax and Bak, seen as a shift to the right in the resulting histogram. We also observed redistribution Bax from cytosol to the mitochondria without changing the protein expression levels of Bax and Bcl-2 after γ-irradiation (Fig. 1B). Confocal microscopy clearly revealed that Bax was translocated to the mitochondria (Fig. 1C). We also showed that Bax and Bak were oligomerized after radiation treatment (Fig. 1D).

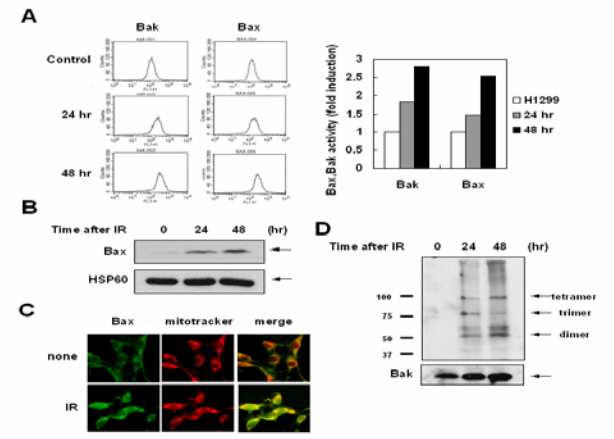

Figure 1. Radiation induces alterations in the conformation of Bax and Bak proteins in human non-small cell lung cancer cells.

To determine the role of p38 MAPK in mitochondrial-activation mediated apoptotic cell death induced by γ-radiation, we pretreated cells with SB203580, p38 MAPK specific inhibitor, or with a dominant negative form of p38 MAPK, and analyzed its effect on radiation-induced cells with investigated the role of p38 MAPK in cytochrome *c* release from mitochondria induced by radiation treatment. As predicted, SB203580 treatment or overexpression of dominant negative p38 MAPK effectively blocked the radiation-induced mitochondrail membrane potential loss (Fig. 2B), cytochrome *c* release and subsequent caspase activation (Fig. 2C). These results indicate that p38 MAPK acts as an important mediator of the radiation-induced cytochrome *c* release from mitochondria. We next studied whether p38 activation is required for the activations of Bax and Bak after radiation treatment. Inhibition of the p38 MAPK pathway by SB203580 or by ectopic expression of a dominant negative p38 MAPK dramatically suppressed

the radiation-induced activity-related conformational changes of Bax and Bak (Fig. 2A), and mitochondrial translocation of Bax proteins (Fig. 2C) in non small cell lung cancer cells.

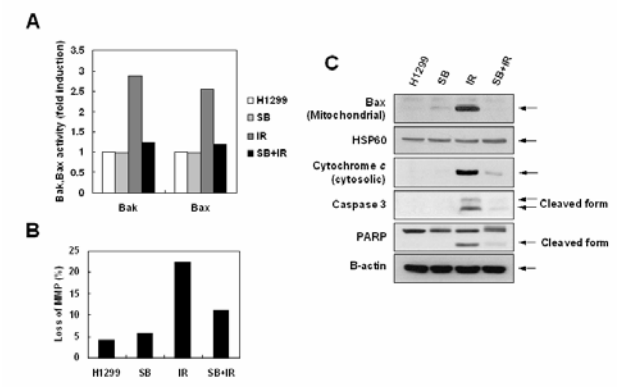

Figure 2. Activation of p38 MAPK is required for the activation-related conformational changes in Bax and Bak.

Treatment of cells with γ-radiation resulted in increase of the PKCδ activity (Fig. 3A). PKCδ activity was apparent at 24 hr, peaked at 48 hr after radiation treatment. Inhibition of PKCδ effectively suppressed the radiation-induced p38MAPK activation, activity-related conformational changes of Bax and Bak, mitochondrial translocation of Bax proteins, and cytochrome *c* release (Fig. 3B and 3C). These findings suggest that activation of PKCδ is necessary for the progression of apoptotic cell death through p38 MAPK-mediated mitochondrial activation pathway after radiation exposure.

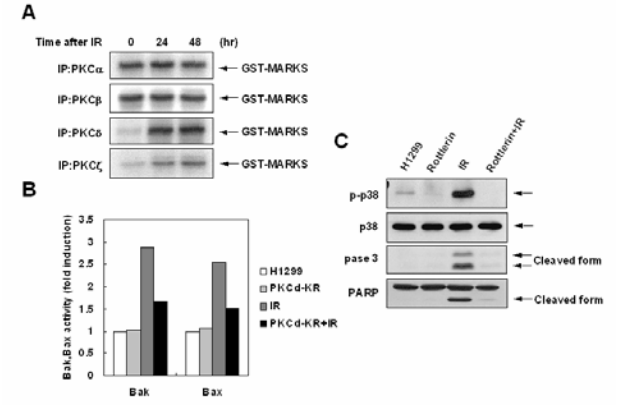

Figure 3. Activation of PKC is required for radiation-induced p38 MAPK activation and conformational changes in Bax and Bak.

It has been shown that PKCδ acts as a lipid-independent enzyme, when it is tyrosine-phosphorylated by Src family kinase. To identify the Src family member responsible for the PKCδ activation during radiation-induced apoptotic cell death, we investigated whether Src activity is stimulated by ionizing radiation in non-small cell lung cancer cells. The kinase activities were measured with enolase or GST-PKCδ as substrates. Fig. 4A shows the time-dependent activation of Src in response to radiation in NCI-H1299 cells. Radiation-induced Src activation was apparently observed after radiation exposure. However, we failed to detect activation of Lyn after irradiation (data not shown). Treatment of PP2, a specific inhibitor of Src kinase efficiently attenuated PKCδ activation and apoptotic cell death seen after irradiation (Fig. 4B and 4C). Moreover, inhibition of Src kinase prevented p38MAPK-mediated Bax and Bak activation, cytochromr c release and caspase activation (Fig. 4D). These results indicate that Src is upstream regulator of PKCδ in radiation-induced apoptotic cell death progression.

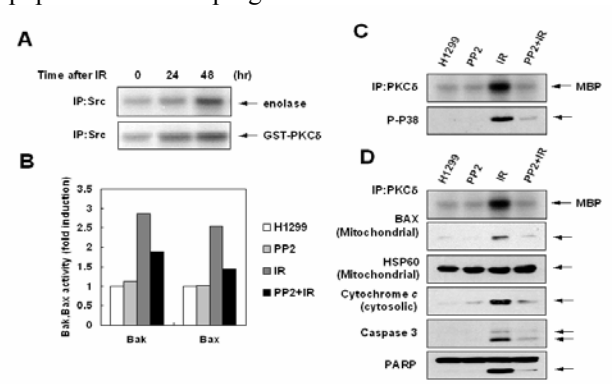

Figure 4. c-Src activation is required for PKCδ activation and subsequent cell death signaling during ionizing radiation-induced apoptotic cell death.

Taken together, these results suggest that Src-PKCδ-p38MAPK signaling plays important role in the activation of Bak and Bax related apoptotic cell death machinery induced by ionizing radiation in human non-small cell lung cancer cells, and could serve as potential targets for strategies that take advantage of signaling-based cell death to enhance tumor cell sensitivity to the radiation therapy.

4. References

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