Cross talk between MAP kinase signalling and apoptotic cell death machinery after γ-irradiation in human cervical cancer cells

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1. Introduction

Exposure of cells to ionizing radiation induces simultaneous activation or down regulation of multiple signaling pathways. These signals play critical role in controlling cell death, cell survival and repopulation after irradiation, in a cell type specific manner. In this study, we investigated the role of MAP kinases in radiation-induced apoptotic cell death/survival signaling in human cervical cancer cells.

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

Ionizing radiation caused induction of caspase-8 activation and Bid cleavage, Bcl-2 phosphorylation/degradation, loss of mitochondrial membrane potential, increase of cytosolic cytochrome *c*, translocation of AIF to the nucleus and subsequent apoptotic cell death in human cervical cancer cells (Fig. 1).

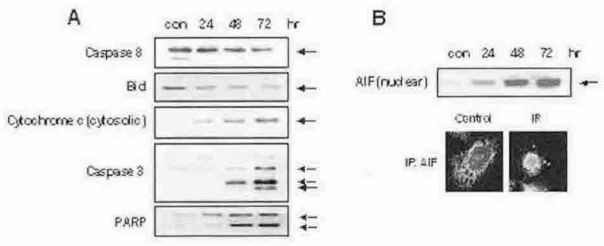

Figure 1. Activation of pro-apoptotic factors after □-irradiation in cervical cancer cells.

We also found that decrease of phosphorylated-ERK1/2 and increase of phosphorylated/activated JNK and p38 MAPK after □-irradiation (Fig. 2). Activation of ERK1/2 by pretreatment with PMA or forced expression of ERK1/2 partially attenuated radiation-induced caspase-8 activation, Bid cleavage, cytochrome *c* release, AIF translocation and apoptotic cell death, indicating that down regulation of ERK is required for the caspase-8-dependent apoptotic cell death pathway.

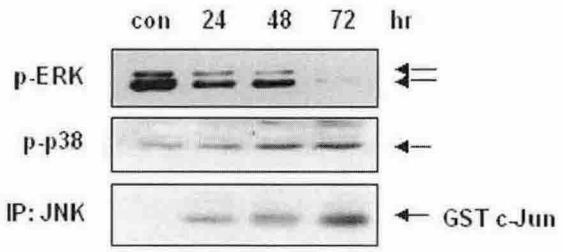

Figure 2. Changes in MAPK activity after □-irradiation in cervical cancer cells.

Inhibition of JNK by pretreatment of SP600125, or by expressing a dominant negative forms of JNK also attenuated loss of mitochondrial membrane potential, increase of cytosolic cytochrome *c*, translocation of AIF to the nucleus. Interestingly, JNK inhibition by both treatments completely attenuated Bcl-2 phosphorylation and degradation. Moreover, expression of phosphorylation site specific-mutant forms of Bcl-2 effectively inhibited phosphorylation and degradation of Bcl-2, mitochondrial membrane potential loss, cytochrome *c* release, AIF translocation and subsequent apoptotic cell death seen after □-irradiation, suggesting that JNK activation is involved in mitochondrial dysfunction-mediated cell death through phosphorylation/degradation of Bcl-2. On the other hand, inhibition of p38 MAPK partially potentiated radiation-induced caspase-8 activation, Bid cleavage and cell death, suggesting that p38 MAPK activation is anti-apoptotic signaling. In this study, we show that down regulation of ERK after irradiation is involved in caspase-8 activation-mediated cell death, and that activation of JNK after irradiation is required for Bcl-2 phosphorylation/degradation and subsequent mitochondrial dysfunction-mediated cell death (Fig. 3). Molecular dissection of the MAPK signaling pathways that regulate the apoptotic cell death machinery is critical for both our understanding of cell survival/death events after ionizing irradiation and development of molecular target for cancer treatment

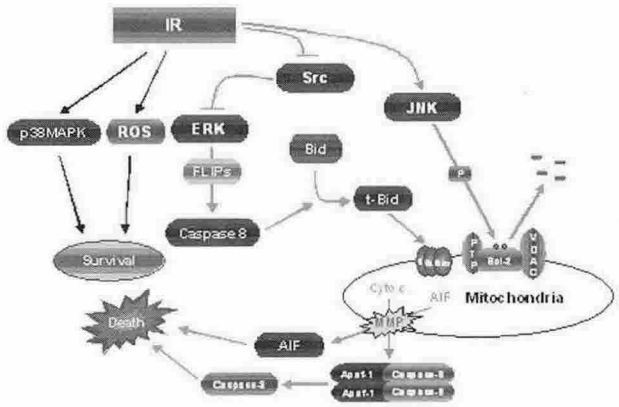

Figure 3. A model for the cross-talk between MAP kinase signaling and apoptosis machinery after □-irradiation in human cervical cancer cells.

4. References

1. Willers H, Dahm-Daphi J, Powell SN. Repair of radiation damage to DNA. Br J Cancer. 2004;90: 297-1301.
2. Wheeler JA, Stephens LC, Tornos C, et al. ASTRO Research Fellowship: apoptosis as a predictor of tumor response to radiation in stage IB cervical carcinoma. American Society for Therapeutic Radiology and Oncology. Int J Radiat Oncol Biol Phys. 1995;32:1487–1493.
3. Coleman CN, Turrisi AT. Radiation and chemotherapy sensitizers and protectors. Crit Rev Oncol Hematol. 1990;10:225-252.
4. Green DR, Reed JC. Mitochondria and apoptosis. Science. 1998;281:1309–1312.
5. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J. 1999;341:233-249.
6. Gottlieb RA. Mitochondria: execution central. FEBS Lett. 2000;482:6-12.
7. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997;91:479-489.
8. Susin SA, Lorenzo HK, Zamzami N, et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature. 1999; 397:441-446.
9. Susin SA, Lorenzo HK, Zamzami N, et al. Mitochondrial release of caspase-2 and -9 during the apoptotic process. J Exp Med. 1999;189:381-394.
10. Sakahira H, Enari M, Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature. 1998;391:96-99.
11. Ferri KF, Jacotot E, Blanco J, et al. Apoptosis control in syncytia induced by the HIV type 1-envelope glycoprotein complex: role of mitochondria and caspases. J Exp Med. 2000;192:1081-1092.
12. Schulz JB, Bremen D, Reed JC, et al. Cooperative interception of neuronal apoptosis by BCL-2 and BAG-1 expression: prevention of caspase activation and reduced production of reactive oxygen species. J Neurochem. 1997;69:2075-2086.
13. Goossens V, De Vos K, Vercammen D, et al. Redox regulation of TNF signaling. Biofactors. 1999;10:145-156.