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Arsenic trioxide synergistically enhances radiation response in human cervical cancer cells through ROS-dependent p38 MAPK and JNK signalling pathway

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1. Introduction

Many factors affect susceptibility of tumor cells to ionizing radiation. Among them intrinsic apoptosis sensitivity or resistancy seems to play an important role. The use of chemical modifiers as radiosensitizers in combination with low-dose irradiation may increase the therapeutic efficacy by overcoming a high apoptotic threshold.

Several recent studies demonstrated additive effects of As_2O_3 with conventional chemotherapeutic agents such as cisplatin, adriamycin, and etoposide, but no synergism. Previously, we have shown for the first time that As_2O_3 sensitize human cervical cancer cells to ionizing radiation. Treatment of As_2O_3 in combination of ionizing radiation has synergistic effects in decreasing clonogenic survival and in the regression of tumor growth in xenografts. We also have shown that the combination treatment enhanced apoptotic cell death through a reactive oxygen species-dependent pathway in human cervical cancer cells.

In this study, we investigated the regulatory mechanism of ROS-mediated mitochondrial apoptotic cell death induced by combination treatment with As_2O_3 and ionizing radiation in human cervical cancer cells.

2. Methods

The combination treatment with radiation and As_2O_3 -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 Conclusions

Since it has been shown that translocation of Bax from the cytosol to the mitochondria causes a decline of mitochondrial membrane potential, we investigated whether the combination treatment with As_2O_3 and γ-radiation induces mitochondrial translocation of Bax. As shown in Fig. 1A, the combination treatment redistributed Bax from cytosol to the mitochondria without changing the protein level, suggesting that activation of Bax involves the combination treatment-induced mitochondrial apoptotic cell death pathway. Since the post-translational modification of Bcl-2, including phosphorylation, has been shown to be associated with its inactivation, and the phosphorylation of Bcl-2 is critically involved in the mitochondrial apoptotic process, we examined whether combination treatment with γ-radiation and As_2O_3 induces Bcl-2 phosphorylation. As shown in Fig. 1B, the combination treatment of HeLa cells induced a marked phosphorylation of Bcl-2, but did not alter Bcl-xL over the time course examined. These results suggest that phosphorylation of Bcl-2 is involved in synergistic enhancement of mitochondrial apoptotic cell death in response to combination treatment with γ-radiation and As_2O_3.

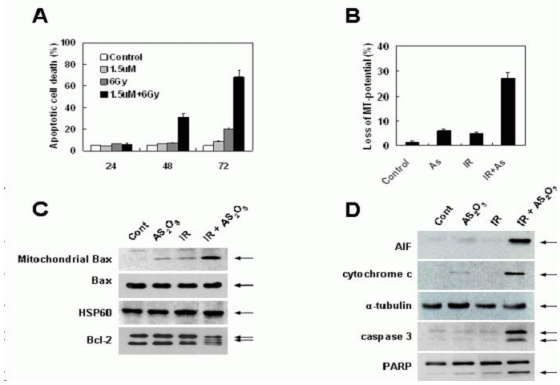

Figure 1. Mitochondrial apoptotic cell death by combination treatment with γ-radiation and As_2O_3.

To investigate a potential involvement of MAPKs in the enhancement of mitochondrial apoptotic cell death induced by the combination treatment with γ-radiation and As_2O_3, we first examined changes in MAPKs activities using immune-complex kinase assay. As shown in Fig. 2A, the combination treatment led to dramatic increase of the p38 MAPK and JNK activities, but did not affect ERK activity. To determine whether activations of p38 MAPK and JNK are involved in the combination treatment-induced apoptotic cell death, we

employed specific inhibitors of p38 MAPK or JNK. Pretreatment of SB203580, a p38 MAPK specific inhibitor, and SP600125, a JNK-specific inhibitor, effectively attenuated the combination treatment-induced apoptotic cell death, while MEK/ERK inhibitor, PD98059, did not (Fig. 2B). Pretreatment of SB203580 and SP600125 also inhibited mitochondrial membrane potential loss (Fig 2C), release of cytochrome *c* and AIF from mitochondria (Fig. 2D) induced by the combination treatment. In addition, p38 MAPK inhibition showed more potent attenuation of mitochondrial apoptotic cell death than JNK inhibition. These results indicate that p38 MAPK and JNK act as important mediators of the combination treatment-induced mitochondrial apoptotic c4ell death in human cervical cancer cells. We further examined whether p38 MAPK and JNK are involved in Bax translocation to the mitochondria and/or Bcl-2 phosphorylation in response to the combination treatment. As shown in Fig. 2E, pretreatment of SB203580, a p38 MAPK specific inhibitor, completely attenuated the combination treatment-induced relocalization of Bax to the mitochondria, and also slightly altered Bcl-2 phosphorylation. On the other hand, treatment with SP600125, a JNK specific inhibitor, suppressed the combination treatment-induced Bcl-2 phosphorylation, but did not affect Bax relocalization (Fig. 2E). In addition, pretreatment of PD98059 did not alter either Bax translocation or Bcl-2 phosphorylation (Fig. 2E). These results indicate that activation of p38 MAPK is specifically required for translocation of Bax to the mitochondria, and both JNK and p38 MAPK are involved in phosphorylation of Bcl-2 in response to combination treatment with γ-radiation and As_2O_3.

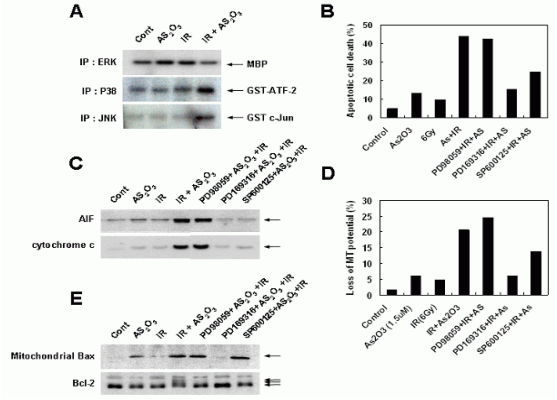

Figure 2. p38MAPK and JNK are required for the combination treatment-induced apoptotic cell death.

We subsequently examined whether ROS induced by the combination treatment is required for p38 MAPK and JNK activations. To determine a linkage between elevation of the intracellular ROS level and p38 MAPK and JNK activations in response to the combination treatment, HeLa cells were preincubated with thiol-containing antioxidant N-acetyl-L-cysteine (NAC) prior to the combination treatment. Pretreatment of NAC completely suppressed the combination treatment-induced p38 MAPK and JNK activations (Fig. 3B) as well as ROS generation (Fig. 3A). In addition, pretreatment of NAC completely attenuated Bax translocation to the mitochondria, Bcl-2 phosphorylation (Fig. 3C), mitochondrial membrane potential loss (Fig. 3D), cytochrome *c* and AIF release (Fig. 3C), and subsequent apoptotic cell death (Fig, 3E). These observations suggest that increase in the intracellular ROS level after the combination treatment is required for p38 MAPK and JNK activations accompanied by the mitochondrial apoptotic cell death.

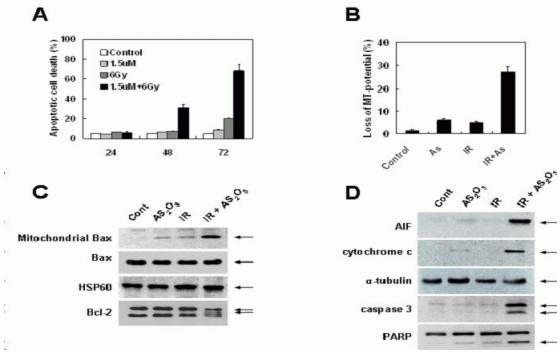

Figure 3. ROS plays a critical role in the activation of p38MAPK and JNK-mediated mitochondrial apoptotic cell death.

Taken together, these results suggest that synergistic enhancement of apoptotic cell death induced by the combination treatment of As_2O_3 with γ-radiation is regulated by two pathways: one is ROS-dependent p38 MAPK activation that mediates Bax redistribution to the mitochondria, and the other is ROS-dependent JNK activation that mediates Bcl-2 phosphorylation.

4. References

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