Effects of trichostatin A on radiosensitivity to high-linear energy transger (LET) radiation in mammalian cells with defects in DNA repair proteins

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In eukaryotes, DNA is associated with histones and packaged into nucleosomes, which are arranged into higher order structures to form chromatin. The chromatin structure contributes to various aspects of DNA metabolism including replication, recombination, and transcription. However, it is still unclear how repair reactions and checkpoint responses caused by heavy-ion irradiation are regulated by chromatin structures. To investigate the roles of chromatin structures in DNA repair after heavy-ion irradiation, we have been focusing on the damage response observed after cells are treated with a potent histone deacetylase inhibitor, trichostatin A (TSA).

To analyze the effects of TSA on repair pathways, we investigated the X-ray sensitivity of wild-type CHO-AA8 cells and CHO mutant lines deficient in homologous recombination (irs1SF cells) [1], non-homologous end-joining (V3 cells) [2], and base excision repair (EM9 cells) [3] in the absence or presence of TSA in a previous study[4]. All three mutant cell lines showed increased X-ray sensitivity (Fig. 1a). TSA treatment enhanced the X-ray sensitivity of wild-type CHO cells. In contrast, TSA enhanced the X-ray radioresistance of irs1SF cells, suggesting that the homologous recombination pathway is involved in radiosensitivity enhancement by TSA. However, TSA did not affect V3 and EM9 survival. These results suggest that non-homologous end-joining and/or base excision repair are stimulated by TSA.

In this study, we investigated argon-ion (LET = 300 keV/μm) sensitivity using the same cell lines. We compared the radiosensitivity of the four cell lines without TSA treatment (Fig. 1a). We found that CHO and V3 cells showed nearly identical dose-response profiles after argon-ion irradiation. We obtained the same results after carbon-ion (LET = 80 keV/μm) irradiation (data not shown), suggesting that non-homologous end-joining is not involved in the repair pathway induced by high-LET ionizing radiation. EM9 and irs1SF cells showed increased sensitivity to argon ions. These results are compatible with those of several recent studies[5,6].

We investigated the effect of TSA on cell line survival (Fig. 1b). TSA treatment enhanced CHO cell sensitivity to argon ions. However, TSA did not affect irs1SF cell sensitivity, which is compatible with the conclusion that non-homologous end-joining is not involved in the repair pathway induced by high-LET irradiation. In contrast, TSA slightly enhanced V3 and EM9 cell radiosensitivity, which seems to be due to the inhibitory effect of TSA on homologous recombination.

Although our survival assay suggests that the non-homologous pathway is not involved in repair after heavy-ion irradiation, we observed that DNA-PK was recruited to the DNA damage sites (data not shown). Currently, we are investigating the localization of repair proteins by indirect immunofluorescence and studying the mechanism underlying the suppression of non-homologous end-joining after heavy-ion irradiation.

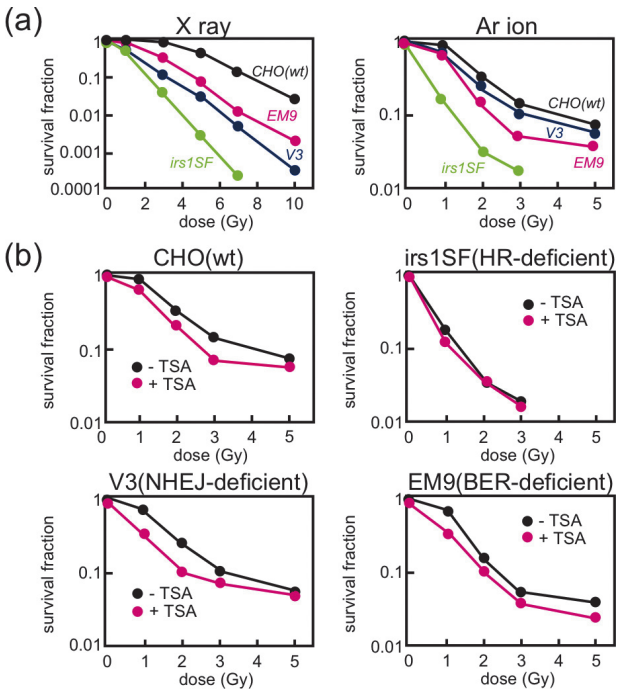

Fig. 1 (a) X-ray and argon-ion sensitivity of CHO, irs1SF, V3, and EM9 cells. Cells were irradiated with X-rays or argon ions (LET = 300 keV/μm), and radiosensitivity was estimated by the clonogenic survival assay. (b) Effects of trichostatin A (TSA) on the radiosensitivity of CHO, irs1SF, V3, and EM9 cells. Cells were pretreated with TSA (0.1 μM) for 10 h and irradiated with argon ions. Subsequently, the cells were cultured for an additional 14 h in the presence of TSA, and radiosensitivity was estimated by the clonogenic survival assay. Abbreviations: wt, wild type; HR, homologous recombination; NHEJ, non-homologous end-joining; BER, base excision repair.

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