CO6-1 Characterization of Clustered DNA Damage Induced by Ionizing Radiation Specifically

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INTRODUCTION: Ionizing radiations wreak critical consequences on all of living organisms, such as individual killing, carcinogenesis, and/or genital disorders. The action mechanism might be based on the DNA damage leading to genetic inability. However, the detailed character of radiation DNA damage, especially the difference between radiation damage and other damage, has been unclear. In the present study, we suppose that clustered DNA damage is a unique radiation damage [1], and estimate quantity and quality of clustered DNA damage induced by various ionizing radiations including gamma-rays and particle beams.

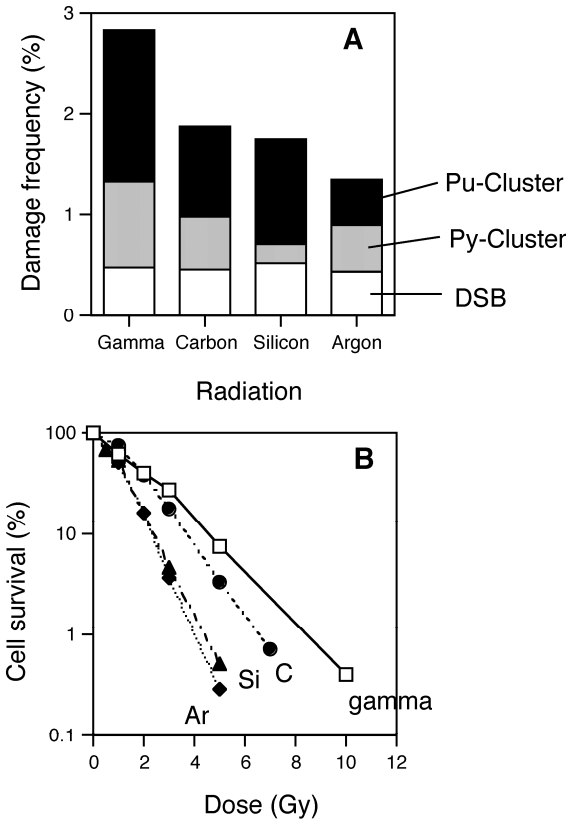
EXPERIMENTS: We ran two experiments for analysis of clustered DNA damage in this study. The first experiment was for the yields of clustered DNA damage in the irradiated culture cells. Chinese hamster ovary (CHO) AA8 cells were irradiated by gamma-rays (0.2 keV/μm), accelerated carbon (13 keV/μm), silicon (55 keV/μm) and argon (90 keV/μm) beams. The irradiated cells were immediately embedded in the agarose plugs. The plugs digested with proteinase were electrophoresed, and the eluted fragments were evaluated for the chromosomal clustered damage. Although double strand break (DSB) were directly evaluated in this manner, the oxidative pyrimidine and purine cluster damage were estimated by additional endonuclease III and Fpg-treatments in the step of gel plug digestion, respectively. At the same time, the cell survival curves for respective radiations were assessed by colony formations.

The second experiment was for the structure of clustered DNA damage induced by various radiations. The complementary double stranded oligodeoxyribonucleotides (TOP : 5'-ttcagcatcgatagcatcctgctcaatcac , BOTTOM : 5'-aagtcgtagctatcgtaggacgagttagtg) were irradiated with gamma-rays, carbon beam and iron beam (200 keV/μm) in 10 mM phosphate buffer (pH 7.6). The irradiated oligonucleotides with radio-labeled at the 5'-ends and modified with aldehyde reactive probe (ARP) at the damaged sites were analyzed by polyacrylamide gel electrophoresis [2]. ^{60}Co-gamma source in KURRI was for gamma-irradiation, and HIMAC in NIRS was for irradiation with other particle beams.

RESULTS: The yields of total chromosomal clustered DNA damage in the irradiated cells showed an inverse proportion for the LET of each radiation (Fig. 1A). Conversely, cell-surviving fractions indicated that radiation effect became elevated in increase of LET of ionizing radiation (Fig. 1B). The result confirms our previous result about the irradiation of extracted DNA molecule targets under abiotic condition [3]. Both these results suggest that the yields of clustered DNA damage do not simply concern the extent of radiation effect. On the other hands, the reason why distribution of respective clustered DNA damage such as DSB and both oxidative base clusters were varied in different radiations has been unclear (Fig. 1A).

The irradiated oligonucleotides showed specific retardations in the electrophoresed gel, corresponding to additive number of ARP as indicating the number of internal damage sites (data not shown).

Fig. 1. Yields of chromosomal clustered DNA damage



in irradiated CHO-AA8 cells (A), and their surviving fractions (B).

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