216 Cell Inactivation and Double Strand Breaks Induction in V79 cells by Monochromatic X rays on Phosphorus K-shell Absorption peak

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Monochromatic X-rays at the energy (2.153 keV) of K-shell absorption peak of phosphorus are strongly absorbed by phosphorus K-shell and followed by Auger processes. We irradiated V79 (Chinese hamster) cells by X-rays of 2.153 keV (peak), and 2.147 keV (low), which is 6 eV lower off the peak. Survival curves revealed that the doses at the survival 10% were 0.83 times less at the peak than at the low; namely, peak X-rays inactivated the cells 1.20 times efficiently. Induction and repair of DSBs were measured with Pulse Field Gel Electrophoresis (PFGE). The percentage of DNA fragments size of below 4.6 Mbp was adopted as measure of DSB amount: the 2.153 keV induced DSBs 1.40 times higher, although the rejoining of induced DSBs were 0.85 times less compare to the 2.147 keV.

217 Localization of Phosphorylated H2AX and Phosphorylated p53 in Cells Inducing X-ray-induced Senescence-like Growth Arrest

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X-irradiation induces permanent cell cycle arrest, named senescence-like growth arrest (SLGA), in cells which have unreparable DNA damage. In this study, we compared localization of phosphorylated H2AX and phosphorylated p53 at Ser15 to determine whether ATM-dependent phosphorylation is involved in SLGA. Phosphorylated H2AX formed several speckle foci in all irradiated cells 30 min after 4 Gy irradiation. Phosphorylated p53 was observed at 30 min and discrete foci were formed 2–4 hrs after irradiation. The number of phosphorylated H2AX foci and phosphorylated p53 foci gradually decreased, but large foci were still remained 24 hrs after irradiation. Those foci were detected at 5 days post-irradiation, when cells began showing SLGA. Both foci co-localized in more than 80% of cells, although not all the foci were co-localized. These results indicate that ATM-dependent phosphorylation is activated in cells inducing SLGA, however, because not all the phosphorylated p53 co-localized with phosphorylated H2AX foci, other factor(s) may play a role in the induction of SLGA.

218 Overexpression of hOGG1 protein enhanced the sensitivity to killing by gamma-rays in HeLa cells

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It's well-known that low LET ionizing radiation, such as X-rays and gamma-rays, produces a unique form of DNA damage called "clustered damages", which is two or more lesions induced within the one or two helical turns of the DNA. *E. coli mutM nth nei* triple mutants were less sensitive to gamma-rays and X-rays than wild-type strain. The triple mutant with plasmid bearing *mutM* gene was more sensitive than wild-type. On the other hand, the triple mutants showed higher sensitivity to H_2O_2 than wild-type. Clustered damages formed by ionizing radiation might be converted to lethal DSB during attempted base excision repair. Recently we found that overexpression of hOGG1 also enhanced the sensitivity to gamma-rays in *E. coli*. In this report we showed HeLaS3 cells transfected by hOGG1 type1a plasmid was more sensitive to gamma-rays than HeLaS3 cells without plasmid. We are now studying the biological effects of clustered damages in human cells.

219 Establishment and characterization of monoclonal antibodies against 2-acetylaminofluorene-DNA adducts

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To develop an in situ detection system of 2-acetylaminofluorene(AAF)-DNA adducts, we tried to establish monoclonal antibodies against them. Mice were immunized with AAF-ssDNA coupled with protein, and then the spleen cells were fused with myeloma cells. Out of 359 hybridoma cells, 5 produced antibodies showing preferential binding to AAF-DNA than to DNA. After clonings, 5 types of monoclonal antibodies were obtained. The antibodies showed the high binding to AAF-DNA, but undetectable or minimal binding to undamaged DNA or UV-irradiated DNA. The competitive inhibition experiments revealed that the epitope is dG-C8-AAF in DNA, but deacetylated dG-C8-AF is also recognized with less efficiency. Using the most promising antibody AAF-1, we could measure the formation of AAF-DNA adducts in DNA of xeroderma pigmentosum group A (XP-A) cells following exposure of N-acetoxy-2-AAF (25–150 uM). Moreover, we could detect AAF-DNA adducts in XP-A cells in situ. Thus, we succeeded in establishing, for the first time, the monoclonal antibodies applicable to in situ detection of AAF-DNA adducts.