Comparison of phosphorylation kinetics in DNA repair proteins after exposure to high and low LET radiations

R. Okayasu (1), A. Okabe (1,2) and K. Takakura (2)

(1) National Institute of Radiological Sciences, Chiba, Japan, (2) International Christian University, Tokyo, Japan (rokayasu@nirs.go.jp /Fax: +81 43-251-4531 / Phone: +81 43-206-3230)

We irradiated plateau phase normal human fibroblasts with 2 Gy X-rays, 70 keV/um carbon (290MeV/n) and 200 keV/um iron ions (500 MeV/n) and observed the kinetics of phosphorylation in various proteins associated with DNA double strand break (DSB) repair. GammaH2AX foci, a marker for DSBs, were detected immediately after irradiation, and the peak of phosphorylation was seen 30 to 60 min post-irradiation for three kinds of radiations. Disappearance of gamma-H2AX foci was much faster for X-irradiated samples than that for heavy ion irradiated samples; the phosphorylation kinetics for carbon and iron ions are similar for gamma-H2AX foci. In contrast, phosphorylation of an NHEJ protein, DNA-PKcs (threonine 2609) was significantly delayed in carbon and iron irradiated cells when compared to X-irradiated cells. Disappearance of DNA-PKcs sites was much faster in X-irradiated samples than carbon and iron samples which showed a similar pattern as in the case of gamma-H2AX. Furthermore, in the case of ATM protein phosphorylation (serine 1981), iron irradiation alone caused a significant initial delay, but the kinetics of disappearance is similar for iron and carbon samples with much higher remaining number of foci in iron samples than those for X-rays and carbon ions.

These results suggest that 1) high LET irradiation induces complex and/or severe DNA DSB damage which affects the function of DSB repair proteins, 2) Both ATM and DNA-PKcs may recognize the complexity of DSBs, but ATM may be more sensitive to detecting the complexity of DSB damage, 3) gamma-H2AX may just recognize DSBs, but not the complexity of them. Our studies also indicate that high LET radiation would be a useful tool to elucidate the complexity of initial DSB damage and the mechanism of repair on these damages.