Research Group for Radiation and Biomolecular Science

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The research objectives of Radiation and Biomolecular Science Group is to elucidate a molecular and cellular process of trans-generational effects by radiation. In JFY2012, we have (1) deterimined the yields of DNA lesions by ion-particle or X-ray irradiations, (2) observed a chromosomal instability induced by DNA damage of non-double-strand-break (non-DSB) type.

Yield of AP sites and other lesions induced in DNA by ion- and X-ray irradiation

In order to obtain the yields of DNA lesions such as single-strand breaks (SSBs), base lesions, and abasic (AP) sites arising in a plasmid DNA molecule irradiated with various ionizing radiations, plasmid DNA (pUC18) was exposed to carbon ions (LET: 13 keV/μm and 60 keV/μm) or X-rays (4 keV/μm) in solutions [1]. Concentrations of Tris in the sample were changed from 0.66 to 200 mM to examine the effect of scavenging capacity. Irradiated samples were then analysed using an enzymatic probe method. We used Nfo, Fpg and Nth proteins to convert the AP sites, purine base lesions, and pyrimidine base lesions into detectable SSBs. At higher concentrations of Tris, the yields of single base lesions were significantly higher than those of single AP sites (Fig. 1). The relative yields of single AP sites were less than 10 % of the total damage produced at a scavenger capacity mimicking that in cells (3×10^8 /s). The dependence of the yield of AP sites on scavenging capacity was similar to that of SSBs. The ratios of the yield of isolated AP sites to that of SSBs induced by carbon ion or X-ray irradiation were relatively constant over the tested range of scavenger capacity. These results indicate that the reaction of water radiolysis products, presumably OH radicals, with the sugar-phosphate moieties in the DNA backbone induces both AP sites and SSB with a constant ratio.

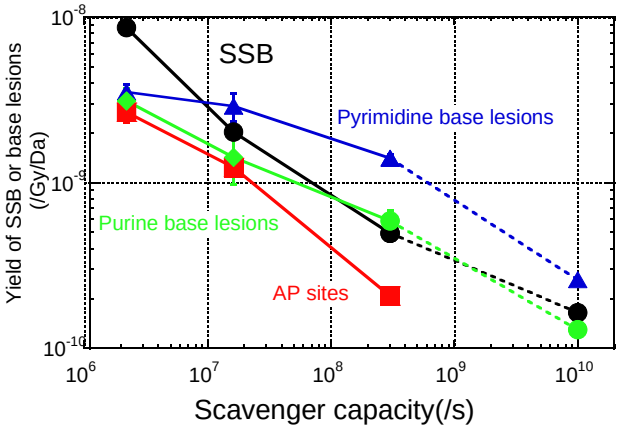

Fig. 1 Dependence of the yield of prompt SSBs or single base lesions on scavenging capacity in the plasmid DNA solution irradiated with C^{6+} ions (13 keV/μm) at 5.6 °C (●: prompt SSB) or after a post-irradiation incubation for 30 min at 37 °C in the presence of Nth (▲: pyrimidine base lesions), Fpg (◆: purine base lesions), or Nfo (■: AP sites). The dotted lines indicate previously reported data [2] shown for comparison.

Induction of chromosomal instability by non-DSB damage

When base lesions, AP sites or SSBs are proximately arising within two DNA helical turns, this site has been called as clustered damage known to be one of the most harmful damage in a cell. The clustered damage sites are categorized into two types, namely DSB and non-DSB type. There have been little evidences how the non-DSB clustered damage is involved in the induction of trans-generational effects so far. To elucidate the role of non-DSB damage, particularly composed of base lesions, in the induction of trans-generational effect, we investigated instability of human chromosome with base lesions transferred into non-damaged mouse cells [3].

A human chromosome exposed to ultra-violet-A (UV-A) light which efficiently induces base lesions such as oxidative guanine was transferred into un-irradiated mouse recipient cells by microcell fusion. We examined the frequency of chromosome aberration induced in the transferred human chromosome using a whole chromosome painting fluorescence *in situ* hybridization (WCP-FISH) method (Fig. 2). The human and mouse chromosome were stained in red and in blue, respectively. The transfer of un-irradiated human chromosome did not cause any effects for both the transferred and recipient mouse chromosomes (Fig. 2c and 2d). In contrast, the transfer of the UV-A irradiated human chromosome enhanced the number of chromosome aberration (Fig. 2b), and chromosome aberrations were observed not only in the human chromosome but also in unirradiated mouse chromosomes (Fig. 2e and 2f). These results suggest that non-DSB damage could induce chromosomal instability even in the un-irradiated recipient mouse cells.

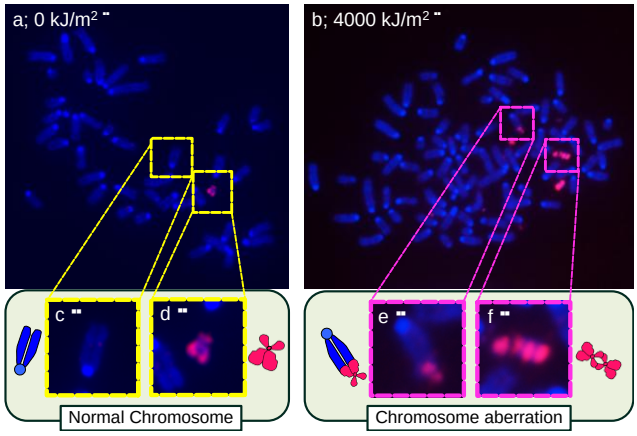

Fig. 2 Mouse chromosomes with un-irradiated human chromosome (a). Normal mouse (c) and human (d) are indicated. Mouse chromosomes with UV-A irradiated (4000 kJ/m^2) human chromosome (b). Delayed chromosome aberrations were observed such as rejoining of the irradiated human and a mouse chromosome (e), or rejoining of the two human chromosomes (f).

References

[1] T. Shiina *et al.*, Radiat. Environ. Biophys. **52**, 99 (2013).
[2] T. Ushigome *et al.*, Radiat. Res. **177**, 614 (2012).
[3] A. Urushibara *et al.*, Mut. Res., submitted.