UV-A damage to DNA causes genetic instability of non-irradiated normal chromosomes

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Ionizing radiation induces a variety of DNA lesions such as chemical modifications or bonding scission. These lesions are thought to be strongly relevant to radiobiological effects, such as mutation induction or cell death. Even when the irradiated cell survive, the progeny of them after a number of cell division is found to frequently undergo chromosome aberrations, so called genetic instability. The instability is likely caused by the malfunction of the cellular activities that maintain genome in normal status. Thus it might play a crucial role in radiation-induced carcinogenesis. Although many studies have been focused the genetic instability with regard to preventing cancer, the underlying mechanism still remains unclear. Recently, localized lesions, so called clustered DNA damage site, consisting of two of more oxidative nucleobase lesions, apurinic/apyrimidinic (AP) sites, or breaks of one of the two strands of a DNA molecule (SSBs), have been known to be strongly related to raidobiological effects. Particularly nucleobase lesions in the clustered damage site play an important role in induction of mutations [1]. In this study we examined how the nucleobase lesions are involved in the genetic instability.

In order to discriminate the effect of nuclobase lesions from that of AP sites or SSBs, we exposed human choromosome 21 in “microcells” to Ultra Violet-A radiation (UV-A; 365nm), which has been known to induce oxidative base lesions. We used a microcell-fusion technique to transfect a specific chromosome with UV-A damage into non-irradiated cells. After UV-A irradiation with a dose of 400 or 4000 kJ/m^2, the microcells were fused to non-irradiated mouse m5S cells (recipient cells). The fused microcell-hybrid cells were cultured in selective medium for about one month, and then, several clones were established. Each clone is originated from an individual cell that has an irradiated human chromosome. The stability of the irradiated and unirradiated human chromosomes and unirradiated mouse chromosomes in the microcell hybrids over 20 population doublings post irradiation were examined by whole-chromosome painting and fluorescence in situ hybridization (WCP-FISH). We used a probe specific for human chromosome 21. Microscopic images were analyzed for both human and mouse chromosomes as shown in Fig. 1.

The ploidy (the number of sets of chromosomes in a cell) of the mouse recipient cells increased, and chromosomal aberrations occurred not only in the UV-A-irradiated human chromosome but also in the non-irradiated mouse chromosomes. The frequencies of these abnormalities increased with the UV-A dose received by the transferred human chromosome. In contrast, in the control experiment, in which an non-irradiated human chromosome was transferred, the micro-cell hybrids remained diploid, and the frequency of chromosomal aberrations in both the transferred human chromosome and recipient mouse chromosomes remained nearly normal. These results indicate that a chromosome harboring oxidative nucleobase damage induced by UV-A irradiation is unstable and transmits instability to chromosomes of non-irradiated recipient mouse cells.

Although the mechanism of the genetic instability of non-irradiated normal chromosomes has still been less understood, certain types of oxidative nucleobase damage might strongly involved in this phenomenon. One of the major nucleobase damage induced by UV-A irradiation is oxidative guanine, 8-oxo guanine. We suggest that candidate target of UV-A radiation in chromosome is likely to be telomere, because it consists of a guanine (G)-rich repeated sequence, TTAGGG, at the terminus of chromosomes. This specific DNA terminal structure does not code any genes, but is thought to play an important role in partition of chromosome pairs during mitosis (cell division). Thus the telomere instability increases the probability of chromosomal instability over many cell divisions rather than prompt cell death. The fused microcell-hybrid cells with destabilized telomere of the human chromosome 21 might undergo incorrect cell division, and finally cause failure of the division resulting polyploidy, or fusion of chromosomes even if they are normal, as observed in this study.

The obtained results that the UV-A damage to DNA significantly affects unirradiated normal chromosomes have a significant impact in the research field of radiation carcinogenesis, as well as in cancer therapy. Details of the incomplete cell division originated from oxidative nucleobase lesions, and mechanism of the chromosome aberrations should be addressed in future studies. Particularly failure of function of cell division machineries, such as centromere formation, will be an important point to be focused.

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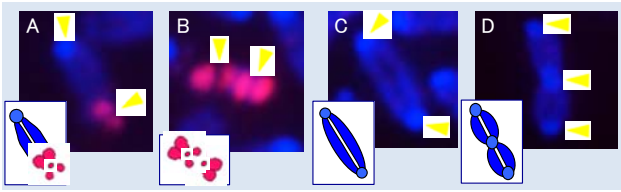

Fig.1 Examples of chromosome aberrations.
Microscopic images obtained as representative delayed chromosome aberrations and their schematic figures (insets) are shown. UV-A irradiated human and unirradiated mouse chromosomes were stained with red and blue dyes, respectively. Arrowheads indicate centromeres. A UV-A irradiated human chromosome fused to an unirradiated mouse chromosome (A). A dicentric chromosome derived from two human chromosomes (B), and two mouse chromosomes (C). A multicentric chromosome (D).

References

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