Induction of genetic instability by DNA damage

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- In order to clarify the cause of genetic instability induced by ionizing radiation, we transferred UV-A induced DNA lesions into unirradiated mouse recipient cells by microcell fusion.
- The microcell hybrids with UV-A induced DNA lesions induced chromosomal instability.
- This result suggests that the UV-A induced DNA damage causes genetic instability in unirradiated cells.

Abstract

Ionizing radiation induces genetic instability in the progeny of irradiated cells. Previous studies suggest that DNA double strand-breaks (DSBs) and subsequent repair processes are involved in the induction of genetic instability. Since it seems unlikely that DSBs persist through several cycles of cell division, we hypothesize that some DNA lesions that remained after repair of DSBs are relevant to the induction of genetic instability. To elucidate whether genetic instability is induced by non-DSBs type of DNA damage (non-DSBs damage), particularly oxidative base lesions, we transferred irradiated chromosome into unirradiated recipient cells by microcell fusion. The transferred chromosomes were analyzed by whole chromosome painting fluorescence *in situ* hybridization (WCP-FISH). The microcell hybrids transferred with Ultra Violet-A (UV-A) irradiated human chromosomes increased their ploidy. In addition, chromosome aberrations occurred not only in the UV-A irradiated human chromosome but also in the unirradiated mouse chromosomes, and the frequencies of these abnormalities were increased depending on the irradiation dose of transferred human chromosomes. These results suggest that the non-DSBs damage induced genetic instability.

1. Background

Ionizing radiation induces various types of DNA lesions such as base damage, strand breaks and DNA-protein cross-links. These lesions have been thought to be strongly relevant to important biological effects, including chromosome aberration, cell death and gene mutation. These abnormalities are not usually observed in irradiated cells, which had survived by repairing DNA lesions. Survived cells of after irradiation should show very similar, or identical, characteristics to unirradiated cells. However, the progeny of survived cells has been found to frequently undergo gene mutations, chromosome aberrations, and cell death [1, 2]. This phenomenon is called genetic instability. The radiation-induced genetic instability is likely caused by the malfunction of the cellular activities that maintain the genome [3], and is considered to play an essential role in radiation carcinogenesis [4]. Therefore, the elucidation of the mechanisms of genetic instability would be highly valuable with regard to preventing cancer. However, the underlying mechanisms remain unclear. One of the reasons of this may lie in the fact that it is extremely difficult to identify the cause of a radiation effect, as potential candidates are quite diverse: multiple types of DNA lesions, such as DSBs, single-strand breaks (SSBs), base damage, and abasic sites, or damage to various proteins, membranes and organelles (Fig. 1). A number of reports suggest that DSBs are likely involved in the induction of genetic instability [5]. However,

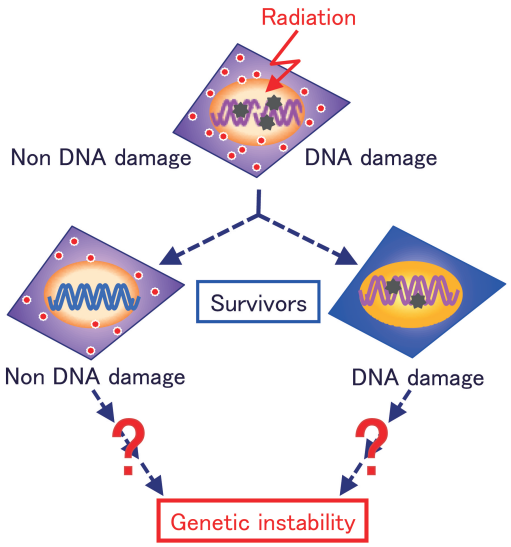

Fig.1. Induction of genetic instability by ionizing radiation
Ionizing radiation induces both non-DNA damage and DNA damage in a cell. These types of damage in surviving cells might induce genetic instability. However, which type of damage causes delayed effect such as delayed chromosome aberration was not known.

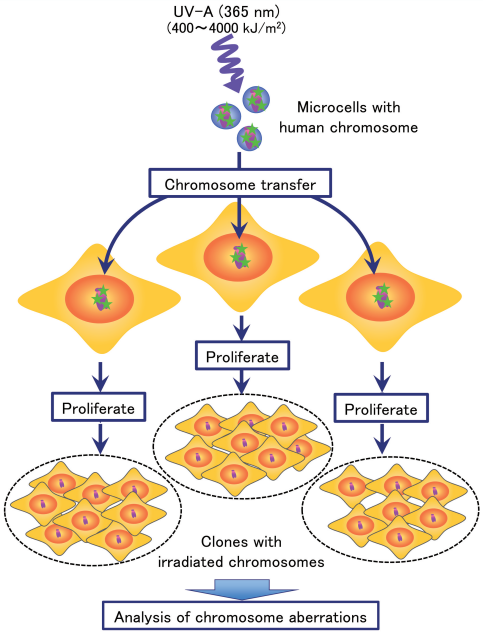

Fig. 2. Transfection of DNA lesions by microcell-mediated chromosome transfer
The human chromosomes were irradiated with UV-A, and introduced into the unirradiated recipient cells by microcell-mediated chromosome transfer. The microcell hybrids establish a clone from a single cell. Chromosome aberrations were scored in each established clone.

cells monitor DSBs quite strictly. It is impossible for cells to undergo dozen times of cell division in the presence of DSBs. Moreover, it has been reported that cells partly deficient in DSBs repair get unstable even with very low amount of DSBs, and are less stable than normal cells [6]. In these cells, DSBs may be repaired with low fidelity, leading to non-DSBs type of repair product (non-DSBs damage). We assumed this non-DSBs damage could lead to genetic instability. This type of damage is called clustered DNA damage, which possesses multiple lesions in very close proximity [7, 8].

2. Outline

To find out whether non-DSBs damage is relevant to ionizing radiation-induced genetic instability, we planned to specifically transfer non-DSBs damage to cells. One can prove whether non-DSBs damage causes genetic instability by measuring the induction of genetic instability in cells that were transferred with DNA damage (Fig. 1). We used microcell-mediated chromosome transfer to transfect exogenous non-DSBs damage (Fig. 2). In microcell-mediated chromosome transfer, a type of cell called “microcell”, which contains chromosomes, is extracted from the donor, and subsequently fused to a recipient cell. Exogenous chromosomes from the donor are thus transferred to other cells (recipient cells). One can find out whether DNA damage leads to genetic instability, by introducing DNA damage, generated in the extracted chromosomes of the microcell, into the unirradiated recipient cells. In the present study, DNA damage was induced by UV with a long wavelength (UV-A; Ultra Violet-A, 365 nm). DNA damage could be also generated by X-rays or γ-rays, which is a well-known inducer of genetic instability. However, X-rays or γ-rays simultaneously generate DSBs, which will prevent one from specifically determining the effect of non-DSBs damage. UV-A induces oxidative base lesions (non-DSBs damage) which could be also generated by X-rays or γ-rays, while inducing few DSBs and few typical UV damage, such as photoproducts and cyclobutane pyrimidine dimers.

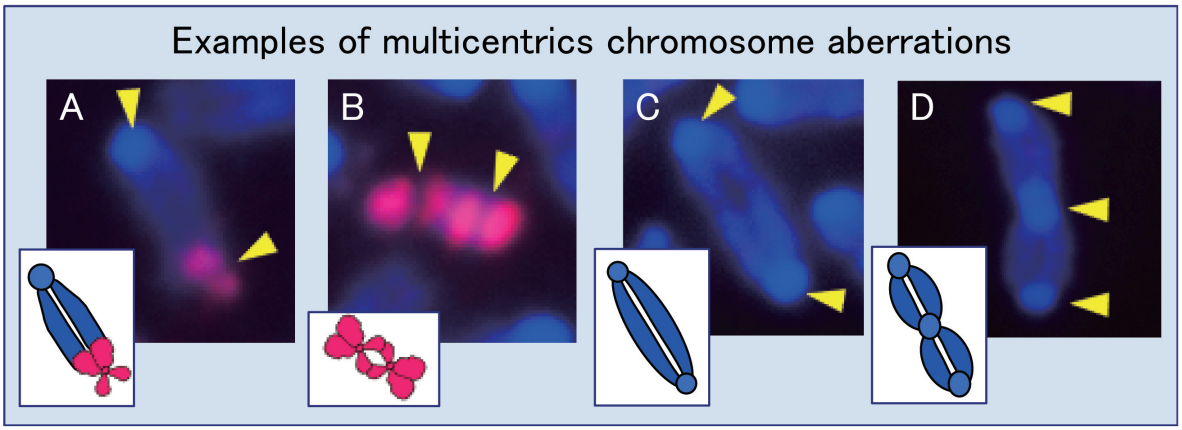

Fig. 3. Examples of chromosome aberrations
These pictures and figures show representative delayed chromosome aberrations. UV-A irradiated human chromosomes were stained with red dyes, and mouse chromosomes were stained with blue dyes. Arrowheads indicate centromeres. (A) A UV-A irradiated human chromosome fused to an unirradated mouse chromosome. (B) A dicentric chromosome formed from two human chromosomes. (C) A dicentric chromosome formed from two mouse chromosomes. (D) A multicentric chromosome.

Thus, UV-A is useful in specifically generating non-DSBs damage. In this study, chromosomes contained DNA damage which were induced by UV-A were introduced in unirradiated recipient cells, and induction of genetic instability was assessed by observing the frequency of delayed chromosome aberrations, in order to evaluate the underlying mechanisms of genetic instability (Fig. 2).

3. Details

Mouse A9 cells containing a human chromosome 21 were used as chromosome donor cells. Mouse m5S cells, which retained near-diploid karyotype, were used as chromosome recipient cells. The human chromosomes were irradiated with 400 or 4000 kJ/m^2 of UV-A, and then a human chromosome was introduced into the unirradiated recipient cell by the microcell-mediated chromosome transfer method. The microcell hybrids were cultured in selective medium, and then, clones each of which derived from a single cell were established. Each clone is originated from an individual cell that has an irradiated human chromosome. The stability of the irradiated human chromosomes and unirradiated mouse chromosomes in the microcell hybrids over 20 population doublings post irradiation were examined by WCP-FISH, using a probe specific for human chromosome 21. We examined the effects of irradiated human chromosomes and unirradiated mouse chromosomes by using this procedure (Fig. 3). We measured delayed chromosome aberrations to assess the induction of genetic instability. The frequencies of chromosome aberrations, such as those in Fig. 3A or 3B that involves UV-A irradiated human chromosomes, were low in clones transferred with unirradiated human chromosomes. In these clones, the frequencies of cells with chromosome aberrations were on average 3 %. In clones that were derived from cells transferred with human chromosomes irradiated with 400 kJ/m^2 and 4000 kJ/m^2, the frequencies of cells with chromosome aberrations increased with increasing dose, and were on average 6.38 % and 50.8 %, respectively. These results indicate that the stability of chromosomes decreases with increasing dose of UV-A.

We also examined mouse chromosomes of unirradiated recipient cells. Interestingly, the frequencies of chromosome aberrations that derived from unirradiated mouse chromosomes but not from human chromosomes (Fig. 3C and 3D) also increased with increasing dose of UV-A. These results suggest that the transfer of UV-A irradiated chromosomes in recipient cells also affects unirradiated chromosomes. To further look at the extent of chromosome instability in unirradiated chromosomes, we scored the total number of chromosomes in cells that were transferred with human chromosomes. A normal

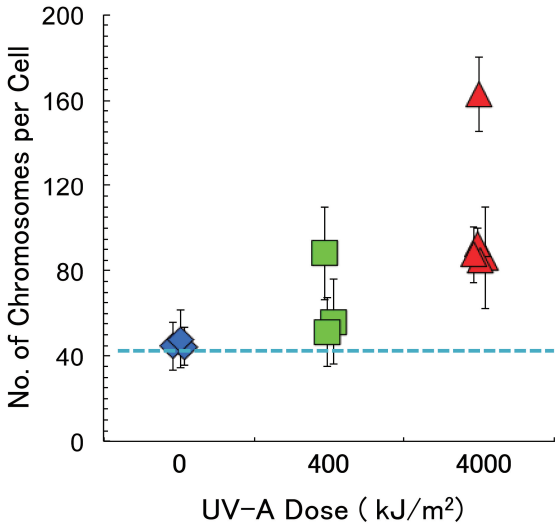

Fig. 4. Analysis of chromosome numbers
A normal microcell hybrid has 43 chromosomes (dashed line). (◆) Cells of a clone transferred with unirradiated human chromosome. (■) Cells of a clone transferred with UV-A irradiated (400 kJ/m^2) human chromosome. (▲) Cells of a clone transferred with UV-A irradiated (4000 kJ/m^2) human chromosome.

cell transferred with a single human chromosome has 43 chromosomes, as recipient mouse cells possess 42 chromosomes. As expected, cells transferred with human chromosomes had approximately 43 chromosomes. However, in cells transferred with UV-A irradiated human chromosomes, the total number of chromosomes increased with increasing dose of UV-A (Fig. 4). These results suggest that the transfer of a chromosome with DNA damage perturbs the homeostasis of the unirradiated recipient cell.

4. Spreading effect and significance of the results

The obtained results that the transfer of non-DSBs damage leads to the induction of genetic instability have a significant impact in the academic field, as the finding of the initial trigger has an important implication to the later events in cells that eventually leads to genetic instability. Thus, our findings have the potential to be applied in preventing the induction of genetic instability, in elucidating the mechanisms of radiation carcinogenesis, as well as in cancer therapy.

5. Future plans

In this study, we found that the trigger of genetic instability is DNA damage. However, the subsequent events that would follow are still unknown. In future, we will investigate how DNA damage results in various delayed effects to elucidate the mechanism of genetic instability. Our future study includes the elucidation of (1) the induction mechanism for abnormal chromosome numbers, and (2) the mechanism of generating chromosome aberrations in unirradiated chromosomes.

References

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Glossary

1. WCP-FISH; Whole Chromosome Painting Fluorescence *in situ* Hybridization
FISH is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. WCP-FISH is painted by hybridizing fluorescent labeled probes for the whole chromosome. The chromosome samples are observed using a fluorescence microscope.

2. Microcell-mediated chromosome transfer method
The method is one of chromosome (gene) transfer techniques. In microcell-mediated chromosome transfer, a type of cell called "microcell" , which contains chromosomes, is extracted from the donor, and subsequently fused to a recipient cell. This method has the advantages of (1) the gene transfer efficiency, and (2) the stability of the transfected gene.