Analysis of complex DNA lesions generated by heavy ion beams

Hiroaki Terato1, Ruri Tanaka1, Yusuke Nakaarai1, Ryoichi Hirayama2, Yoshiya Furusawa2, and Hiroshi Ide1

1Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan and 2Heavy-Ion Radiobiology Research Group, Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, 4-9-1 Anagawa, Chiba 263-8555, Japan

ABSTRACT

Ionizing radiation induces clustered DNA damage, which contains localized multiple lesions in duplex DNA molecules. It has been thought that due to complex nature, clustered DNA damage is refractory to repair or associated with error-prone repair and results in severe biological endpoints as compared to sparsely-distributed lesions. High linear energy transfer (LET) radiations such as heavy ion beams exert a greater relative biological effect (RBE) than low LET radiations such as X- and gamma-rays. In the present study, we analyzed the yields of clustered DNA damage produced by high and low LET radiations using in vitro and in vivo systems and examined whether the differential formation of clustered DNA damage accounts for distinct RBE values of these radiations.

INTRODUCTION

Ionizing radiation causes damage to DNA in living organisms and induces mutagenic and/or lethal events. When ionizing radiation traverses target cells, it deposits energy onto bio-molecules and water along the track and causes ionization of these molecules. Ionization of DNA molecules generates damage to DNA directly, whereas that of water results in the formation of highly reactive species such as hydroxyl radicals, hydrogen atoms, and hydrated electrons. These species diffuse to react with proximal DNA constituents, and hence generating DNA damage in an indirect manner. However, regardless the direct or indirect action of ionizing radiation, at least part of lesions induced by ionizing radiation is localized on the DNA strand. This type of site containing localized multiple lesions is referred to as clustered DNA damage (1, 2). It is possible that owing to complex nature, clustered DNA damage is refractory to repair or associated with error-prone repair and results in severe biological consequences as compared to sparsely-distributed lesions (3). A double-strand break (DSB), comprising two proximal single-strand breaks (SSBs) on opposing strands, is a typical clustered DNA. The cells deficient in DSB repair is hypersensitive to ionizing radiation (4), supporting the crucial role of clustered DNA damage in the biological effect of ionizing radiation.

The use of DNA repair enzymes such as DNA glycosylases and AP (apurinic/apyrimidinic) endonucleases expanded the spectrum of experimentally-detectable clustered DNA damage. These enzymes convert damaged bases and AP sites into SSBs. If a multiply damaged site contains two or more lesions of damaged bases and/or AP sites (clustered base damage), the site can be converted to an experimentally-measurable DSB upon treatment with DNA glycosylases or AP endonucleases (5). This also suggests that if similar reactions occur during the base excision repair of clustered base damage in cells, the site of clustered base damage is converted to a lethal DSB, and hence leading to abortive repair (Figure 1).

Figure 1. Abortive repair of clustered base damage.

Although the yields of clustered DNA damage including DSBs and clustered base damages by various types of ionizing radiation have been measured in previous studies (5), these results were conflicting regarding whether high LET radiations give rise to more clustered DNA damage.
than low LET radiations. Thus, the mechanism by which high LET radiations brings about elevated biological effects still remains elusive. In the present study we analyzed the yields of isolated and clustered DNA damage induced by high and low LET radiations using in vitro and in vivo systems, and examined the correlation between the yield of DNA damage and the LET value.

RESULTS AND DISCUSSION

In the in vitro analysis of clustered DNA damage, we used two types of DNA: circular pDEI.19 plasmid DNA (4,814 bp) and linear lambda phage DNA (48,502 bp). DNA substrates were dissolved in Tris buffer and irradiated with gamma-rays (LET = 0.2 keV/μm) and accelerated carbon (13 keV/μm) and iron (200 keV/μm) ions. Part of irradiated samples was digested by appropriate DNA glycosylases for the detection of base damage (6). The conformational changes of plasmid DNA were analyzed by conventional agarose gel electrophoresis to quantitate isolated and clustered DNA damage. With lambda DNA, damaged bases including AP sites were quantified with the aldehyde reactive probe assay (7) and the yield of DSBs was analyzed from the number average length of DNA determined by pulse field gel electrophoresis (8).

In the present study, we classified clustered DNA damage into three categories: DSBs, oxidative pyrimidine cluster, and oxidative purine cluster. The yields of individual type of radiation-induced clustered DNA damage were 2–8 sites per 10^4 bp per Gy in plasmid DNA (Figure 2). Interestingly, the yields of clustered DNA damage decreased with increasing LET values of radiation (damage yield: gamma > carbon > iron). The yields of individual type of isolated damage (i.e., SSBs, oxidative pyrimidines, and oxidative purines) in the plasmid substrate were 1–4 sites per 10^4 bp per Gy and as with clustered DNA damage, decreased with increasing LET values of radiation. Similar results were obtained in the irradiation of lambda DNA.

For analysis of the in vivo formation of clustered DNA damage, Chinese hamster ovary (CHO) cells were irradiated with gamma-rays and accelerated carbon and iron ions. Irradiation was performed at 4 °C to minimize DNA repair. Irradiated cells were embedded in agarose gel plugs, digested by proteinase K, and subjected to agarose gel electrophoresis (9). After electrophoresis, the amounts of DNA retained in and released from the agarose plug were measured to evaluate DSBs. Upon 10 Gy of irradiation, the fractions of DNA released from the plug were 28%, 16% and 10% for gamma-rays, carbon ions, and iron ions, respectively. This result indicates that as with in vitro irradiation, the yield of clustered DNA damage (DSBs) also decreases in vivo with increasing LET values of radiation.

The present in vitro and in vivo data consistently show that the yield of clustered DNA damage decreases with increasing LET values of radiation, indicating that the yield of clustered DNA damage does not simply account for the greater biological effect of high LET radiations than low LET radiations. The present results also suggest that other factors, such as radiation quality-dependent molecular complexity of clustered DNA damage and abortive or error-prone repair, need to be considered to delineate the underlying mechanism for the elevated biological effect of high LET radiations.

Figure 2. Yields of clustered DNA damage in plasmid DNA irradiated with gamma-rays, carbon ions (C), and iron ions (Fe). Open, hatched, and closed columns indicate DSBs, oxidative pyrimidine clusters, and oxidative purine clusters, respectively.

REFERENCES


*Corresponding author. E-mail: hterato@hiroshima-u.ac.jp