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Carbon ions Versus γ-Irradiation: The Telomeric Effect in Cancer Cells

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Summary

The higher biological effect of Carbon ions hadrontherapy (C+) is explained by the nature of the DNA damages. It is known that cell response to γ-irradiation (γ-IR), but not to C+, is correlated with telomere length in different type of cancer cells. Here, we propose that this “telomeric effect” must result from an effect of ROS in γ-IR compared to C+.

Keywords: Telomere; LMDS; Oxidative Stress; Telomere-induced Foci

Introduction

Carbon ions (C+) hadrontherapy is an alternative treatment for radio-resistant tumors. Compared with photon irradiation (γ-IR), C+ has demonstrated a higher Relative Biological Efficiency (RBE) in vitro [1] and in vivo [2]. Clustered DNA lesions called Locally Multiply Damaged Sites (LMDS) are more difficult to repair and are therefore responsible for the higher RBE of C+ irradiation [3]. In contrast, isolated double strand breaks (DSBs) are thought to be the primary determinant of cell death after γ-IR with a minor implication for LMDS [4]. However, the genomic region damaged by these types of irradiation can also influence the RBE. Telomeres, the heterochromatin structure located at the end of chromosomes, play different roles in response to C+ and γ-IR [5]. While the initial length of telomeres influences cellular responses only to γ-IR, it is subsequent increase in telomeric length that is specifically observed after C+ [6]. To further characterize the initial telomeric effect, we have studied the telomeric DSBs known as Telomere-damage-Induced Foci (TIF) after C+ and γ-IR which yield the same RBE.

Methods and Materials

U373MG cell line (telomere length 14 kb) was a kind gift from Pr. Verelle (EA3846, Clermont-Ferrand, France). C+ irradiation was given at 72 MeV/u, LET 33.6 keV/µm, and X-ray beam of 250 kV at 2Gy/min-1 for photon irradiation. The RBE value for the carbon ion beam relative to X-ray at the D10 levels was determined (Figure A) and used to irradiate cell at the same biologic dose (fold 2). TIFs were determined by immunofluorescence analysis of a DSBs signalizing protein 53BP1 (Novus
biological: NB100-305) and the telomeric protein TRF1 (Abcam: ab10579) co-localization counting. Antibody were used at a dilution of 1:300. Acquisition was done with a Z-stack (Step 0.1µm). At least 30 cells with more than one TIF were counted by condition. Only cells with more than one TIF were considered. The values represent the mean ± s.d of two independent experiments. The clonogenic survival assays were done as previously published [5].

Results and Discussion

We observed no difference in the number of initial DSBs produced directly at telomeric sites between the two types of irradiation (2.46 TIFs per cell at 30min) (Figure B).

However, the percentage of cell with TIF and the average number of TIF per cell increased and reached a peak 6h post-irradiation after γ-IR (3.3 and 86%, Fig. B, C). γ-IR is a well-known inducer of reactive oxygen species (ROS). Due to their GGG stretch, telomeres produce an excess of 8-oxoguanines, compared to the rest of whole genome, when exposed to ROS [7]. This type of DNA damages is poorly repaired at telomeres and frequently turned into single and double strand breaks [8, 9]. Furthermore, it is described in the literature that C+ produced less oxidized purine that γ-IR [10]. Thus, this could explain the appearance of DSBs resulting from the mis-repaired 8-oxoguanine at telomeres.

However, after C+ irradiation, the average number of TIFs per cell (Figure C) and the number of cells impacted by TIF remained lower and were quite stable over the 24h post-irradiation period. It is noteworthy that C+ produces clusters of DNA damage in the vicinity of the track of the particle (see the large 53BP1 foci, Figure D) in a ROS-independent manner [11]. In this context, telomeric damages induced by the impact of the particle are expected to be yield into much complex LMDS. Because of their complexity, LMDS are rarely repaired in accordance with the stable level of TIFs that we have observed over a 24h time period (2.6 to 2.2).

Finally, we observed a higher percentage of cells with residual DNA damages 24h post-γ-IR at telomeres, in comparison with C+ (72% versus 33%), while the mean number of TIF per cells was similar (2.7 versus 2.5). Residual/unrepaired DNA damages are known to promote cell death [12].

Considering that we used doses of γ-IR and C+ leading to the same level of cell death, this observation confirms that residual telomeric damages play a minor role after C+ irradiation. Persistent Telomere-Associated DNA damage Foci (TAFs) are also predictive of an increased risk of secondary cancer [13]. The higher level of TAFs after γ-IR as compared with C+ was concordant with a higher rate of recurrence after γ-IR [2].

Figure 1. Telomeres are targeted differently by photon and carbon irradiations. (A) Clonogenic survival assays were performed after photon or C+ irradiation. The values represent the mean ± s.d of three independent experiments. (B) Representation of the percentage of cells presenting TIFs after 2Gy or 1Gy, respectively with photon and carbon irradiation. The values represent the mean ± s.d of two independent experiments. (C) Average number of TIFs per cell after 2Gy or 1Gy, respectively with photon or carbon irradiation. (D) Representative pictures of TIFs staining, 6h post-irradiation, nuclei are delimited by a white line and TIF is represented by a white arrow.

Globally, these data are concordant with the hypothesis that the initial “telomeric effect” results from a pan-ROS prominent effect of γ-IR, while C+ irradiation acts mainly by LMDS.

Conflict of Interest

None

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