DNA Repair 25 (2015) 54–59

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Brief communication

Enforced telomere elongation increases the sensitivity of human tumour cells to ionizing radiation

Jennifer Fairlie [a], Lea Harrington [a,b,*]

[a] Wellcome Trust Centre for Cell Biology and Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Mayfield Road, EH9 3JR Edinburgh, UK
[b] Institute for Research in Immunology and Cancer, Department of Medicine, University of Montreal, 2950 chemin de Polytechnique, Montreal, Canada H3T 1J4

ARTICLE INFO

Article history:
Received 20 August 2014
Received in revised form
13 November 2014
Accepted 17 November 2014
Available online 24 November 2014

Keywords:
Telomeres
Telomerase
Human tumour cells
Gamma irradiation
DNA damage

ABSTRACT

More than 85% of all human cancers possess the ability to maintain chromosome ends, or telomeres, by virtue of telomerase activity. Loss of functional telomeres is incompatible with survival, and telomerase inhibition has been established in several model systems to be a tractable target for cancer therapy. As human tumour cells typically maintain short equilibrium telomere lengths, we wondered if enforced telomere elongation would positively or negatively impact cell survival. We found that telomere elongation beyond a certain length significantly decreased cell clonogenic survival after gamma irradiation. Susceptibility to irradiation was dosage-dependent and increased at telomere lengths exceeding 17 kbp despite the fact that all chromosome ends retained telomeric DNA. These data suggest that an optimal telomere length may promote human cancer cell survival in the presence of genotoxic stress.

1. Introduction

The acquisition of telomerase reverse transcriptase (*TERT*) expression and its ability, together with the telomerase RNA, to maintain telomeres at chromosome ends is one of the hallmarks of cancer [1]. In the absence of telomerase function or upon enforced telomerase inhibition, telomere erosion eventually results in a telomere-specific DNA damage response that leads to chromosome end resection, loss, or fusion, and anaphase bridges (reviewed in [2,3,4]). Short telomeres, which are typical of human cancer cells [5,6], are a preferential substrate for telomere elongation by telomerase in many model systems. Indeed, the levels of the telomerase components TERT and its integral RNA, hTR, are limiting in many cell types including cancer cells (reviewed in [7]). Conversely, long telomeres inhibit access by telomerase, and in some instances excessively elongated telomeres are actively trimmed by complex recombination mechanisms [8–12] (reviewed in [13,14]). Thus, in many cell types, the homeostatic balance between these processes ensures that telomere length is maintained around a given equilibrium telomere length. We questioned why tumour cells tend to maintain short average telomere lengths, and whether long telomeres might be disadvantageous for cell survival in the presence of DNA damage.

2. Materials and methods

2.1. Cell culture and cell irradiation

Cell culture and population doubling level (PDL) calculations were performed as described [15]. Irradiation experiments were carried out according to Boyd et al. [16]. Cells were seeded in 25 cm^2 flasks at a concentration of 1×10^5 cells/flask. After 24 h adherent cells were irradiated at a range of doses from 0 to 10 Gy using a ^{60}Co irradiator, returned to a 37 °C incubator for 24 h, then resuspended in 0.5% w/v trypsin solution and counted on a haemocytometer. Cells were plated in triplicate at 2.5×10^3 per 75 cm^2 flask and incubated at 37 °C for 7–10 days. Cells were fixed and stained with 10% w/v crystal violet in methanol. Clusters of approximately 50 or more

Abbreviations: ALT, alternative lengthening of telomeres; ANOVA, analysis of variance; CST, CTC1, STN1 and TEN1 complex; DSB, double-stranded DNA break; hTR, human telomerase RNA; PDL, population doubling level; PE, plating efficiency; Q-FISH, quantitative fluorescence in situ hybridization; SF, survival fraction; SFE, signal-free end; SV40, simian virus 40; TERT, telomerase reverse transcriptase; TRF, telomere restriction fragment.

* Corresponding author at: Institute for Research in Immunology and Cancer, Department of Medicine, University of Montreal, 2950 chemin de Polytechnique, Montreal, Canada H3T 1J4. Tel.: +1 514 343 6729.
E-mail address: lea.harrington@umontreal.ca (L. Harrington).

http://dx.doi.org/10.1016/j.dnarep.2014.11.005

cells were scored as a single colony. The SF was calculated by determining the plating efficiency (PE, defined as the number of colonies divided by the number of cells seeded), with the first PE value at 0 Gy (of $n = 3$) normalized to 1.0. Thus, the average SF at 0 Gy is near (but not exactly equal to) 1.0, and the SF of cells exposed to irradiation are expressed relative to the SF at 0 Gy. All the cell lines in each graph were subjected to irradiation and subsequent manipulations at the same time. Statistical analysis was carried out using Prism 5.0 (GraphPad, Inc.), in which the SF values (at least $n = 3$) of a cell line at a given irradiation dosage were assigned to a single column (e.g. in Fig. 2A, left, there were 12 columns, representing SF values for 17 PDL and 146 PDL lines at 0, 2, 4, 6, 8, and 10 Gy, respectively). All columns were compared simultaneously using one-way ANOVA (parametric, unmatched observations) followed by a Tukey post-test. The calculated p-values shown represent level of significance (** $p < 0.01$; *** $p < 0.001$) of a given PDL at a particular irradiation dosage relative to the earliest PDL at the same irradiation dosage.

2.2. Telomere length measurements

Q-FISH analysis and Southern blots to assess telomere length were performed as described previously [15]. Q-FISH analysis employed an automated Metafer Slide Scanning platform (Metasystems, Inc.) capable of automated analysis of 8 slides under identical conditions. Unless otherwise stated, Q-FISH profiles shown in the same figure panel were analyzed simultaneously in this manner. Average telomere lengths at a given PDL were determined via linear regression analysis of average telomere signal intensity obtained via 3 independent Q-FISH measurements verified against TRF length determined by southern blot where a.u. (y) were converted to kbp (x) using the formula $y = 64x - 106$. This linear regression analysis yielded an R-squared co-efficient of 0.97 (data not shown). In Fig. 1C, signal-free ends were defined as chromosome ends that yielded no detectable telomere fluorescence signal, from a total of 920 ends analyzed for each sample; the data from one representative experiment is shown ($n = 3$). p-values of $p < 0.05$ (*) or $p < 0.0001$ (****) were calculated using Fisher's exact test (Prism 5.0, GraphPad Inc.).

3. Results

3.1. Prolonged TERT expression and its effect upon telomere length and integrity

In a previous study, we generated and characterized human tumour cell lines derived from a human embryonic kidney parental line containing the SV40 early region and oncogenic Ras, that either expressed exogenous *TERT*, possessed telomerase activity, and underwent telomere elongation (*TERT*-positive) or had *TERT* excised, lost telomerase activity, and underwent telomere erosion (*TERT*-excised) [17]. *TERT*-excised cells remained viable and capable of tumour formation for prolonged periods, and succumbed to apoptosis only upon significant accumulation of telomere signal-free ends [17]. In this study, we separately propagated these cell lines with or without *TERT*, in order to enable an assessment of relative DNA damage sensitivity of clonal isolates that were derived from the same parental line. In *TERT*-positive tumour cells between population doubling levels of 11 and 169, the mean telomere length continuously increased from 12 kbp to 24 kbp (Fig. 1A). Telomere elongation was assessed in two independently derived *TERT*-positive clones and telomere length increased by 54–76 bp per PDL (Fig. 1A and B, data not shown). In two other clonal lines, *TERT* was excised and the rate of telomere erosion ranged between 35 and 38 bp per PDL (Fig. 1A and B, data not shown). As expected, the frequency of telomere signal-free ends correlated positively with the acquisition of very short telomeres (Fig. 1C and D). *TERT*-positive cells, on the other hand, exhibited a statistically significant decrease and eventual elimination in the incidence of SFE as average telomere lengths increased (Fig. 1C and D). *TERT*-positive cells at late passages also did not exhibit evidence of extensive telomere trimming, as judged by the absence of accumulation of shorter telomeres (Fig. 1A: e.g. compare Q-FISH profiles in Vec-1 line at PDL 11 and 169).

3.2. The impact of telomere elongation upon survival after gamma irradiation

We tested the impact of telomere length on the response to ionizing radiation in two independently generated *TERT*-positive and *TERT*-excised cell populations. Without irradiation, all cell types exhibited an equivalent ability to form colonies regardless of telomere length or telomerase status (Fig. 2A, data not shown). Also as expected, at high irradiation doses (8–10 Gy) all cell types underwent significant cell death, with a low or zero survival fraction (SF) (Fig. 2A and B). After exposure to intermediate dosages of irradiation (2–6 Gy), *TERT*-excised cell populations with short telomeres exhibited a significant and dosage-dependent decline in SF relative to the same cell line with longer telomeres (Fig. 2A and B, right; compare blue lines at increasing PDL). The decreased survival of cells with short telomeres after exposure to gamma radiation is in keeping with previous studies showing that short telomeres and DNA damaging agents act synergistically to induce apoptosis [18–25]. However, cell populations with telomeres over 17 kbp in length also demonstrated a statistically significant decrease in SF at intermediate doses of gamma irradiation (Fig. 2A and B, left; compare orange lines at increasing PDL). Specifically, two independently derived *TERT*-positive cell clones with TRF lengths of approximately 24 or 20 kbp (Fig. 2A; 153 and 146 PDL, respectively) exhibited a significant decrease in SF after exposure to 4 or 6 Gy irradiation, compared with the same clones with TRF lengths of 12 or 13 kbp (Fig. 2A; 11 and 17 PDL, respectively). This observation was reproduced in another independent experimental series, where *TERT*-positive populations (PDL 58, 17 kbp; or PDL 142, 20 kbp) exhibited a statistically significant decrease in SF at 4 Gy or 6 Gy compared to the same population with shorter telomeres (PDL 15, 13 kbp) (Fig. 2B). This difference did not appear to be a function of the inherent ability to respond to DNA damage, since we observed similar levels of 53BP1 and γ-H2AX foci in untreated cells and in foci induction after one hour of exposure to 10 Gy in all *TERT*-positive lines, regardless of telomere length (data not shown). A plot of survival fraction against average telomere length demonstrated that average telomere lengths of >12 kbp or <17 kbp yielded a maximal SF across a range of sub-lethal irradiation doses using two independently derived datasets (Fig. 2C and D derived from data in Fig. 2A and B, respectively). This data demonstrates that an optimal range of telomere lengths was associated with an increased resistance to irradiation.

4. Discussion and conclusions

The mechanisms that lead to an increased irradiation sensitivity of cells with very long telomeres may be different than cells with very short telomeres. In primary cells where telomeres are critically short and thus possess signal-free ends, the presence of telomerase activity – accompanied by telomere elongation – rescues cells from the deleterious effects of ionizing radiation [26]. Similarly, the reactivation of telomerase and telomere elongation in a murine cancer model with critically short telomeres leads to rapid tumour progression [27,28], and *TERT* promoter mutations have