Telomere Length Abnormalities in Mammalian Radiosensitive Cells¹

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Abstract

Telomeres are essential functional elements of the eukaryotic chromosomes responsible for chromosome stability maintenance (1). In yeast, telomeres play a role in cellular responses to DNA-damaging agents such as IR,¹ a potent inducer of DNA DSBs. DSB repair proteins, including Ku, are permanently present at yeast telomeres (2), and they relocate to the site of DSB after exposure to IR (3, 4). Although it is not clear whether telomeres have the same role in other organisms, several lines of evidence indirectly link telomere maintenance and cellular response to IR in organisms as diverse as Caenorhabditis elegans and humans (see below). The first line of evidence is provided by the studies involving the human genetic disorders AT and NBS. AT patients show sensitivity to IR, a high incidence of cancer (5), and a number of telomere-associated defects (6–8). Similarly, NBS cells are radiosensitive, and the NBS protein is present at telomeres (9). The second line of evidence is provided by mouse studies. Radiosensitive severe combined immunodeficiency mice deficient in DNA-PK, an enzyme complex consisting of Ku and DNA-PK catalytic subunit that is involved in V(D)J recombination and DSB repair, have abnormally long telomeres and a high frequency of TGFs (10, 11). Similarly, radiosensitive Ku-deficient mice have a defect in telomere function as shown by high incidence of TF (12). In addition, mice deficient in PARP are radiosensitive (13) and show telomere shortening accompanied by TF (14). The third line of evidence is provided by a study of the C. elegans cell cycle checkpoint mutant, mrt-2. This mutant has critically short telomeres and high levels of TF, and it is hypersensitive to IR (15). Taken together, these observations point to the possibility that telomeres may play a role in cellular, or even organismal, responses to IR. To test this possibility more directly, we examined chromosomal radiosensitivity and telomere length in normal mouse cells, in mouse lymphoma cell lines, and in human lymphocytes originating from breast cancer patients.

Introduction

Telomeres are essential functional elements of the eukaryotic chromosomes responsible for chromosome stability maintenance (1). In yeast, telomeres play a role in cellular responses to DNA-damaging agents such as IR,¹ a potent inducer of DNA DSBs. DSB repair proteins, including Ku, are permanently present at yeast telomeres (2), and they relocate to the site of DSB after exposure to IR (3, 4). Although it is not clear whether telomeres have the same role in other organisms, several lines of evidence indirectly link telomere maintenance and cellular response to IR in organisms as diverse as Caenorhabditis elegans and humans (see below). The first line of evidence is provided by the studies involving the human genetic disorders AT and NBS. AT patients show sensitivity to IR, a high incidence of cancer (5), and a number of telomere-associated defects (6–8). Similarly, NBS cells are radiosensitive, and the NBS protein is present at telomeres (9). The second line of evidence is provided by mouse studies. Radiosensitive severe combined immunodeficiency mice deficient in DNA-PK, an enzyme complex consisting of Ku and DNA-PK catalytic subunit that is involved in V(D)J recombination and DSB repair, have abnormally long telomeres and a high frequency of TGFs (10, 11). Similarly, radiosensitive Ku-deficient mice have a defect in telomere function as shown by high incidence of TF (12). In addition, mice deficient in PARP are radiosensitive (13) and show telomere shortening accompanied by TF (14). The third line of evidence is provided by a study of the C. elegans cell cycle checkpoint mutant, mrt-2. This mutant has critically short telomeres and high levels of TF, and it is hypersensitive to IR (15). Taken together, these observations point to the possibility that telomeres may play a role in cellular, or even organismal, responses to IR. To test this possibility more directly, we examined chromosomal radiosensitivity and telomere length in normal mouse cells, in mouse lymphoma cell lines, and in human lymphocytes originating from breast cancer patients.

Materials and Methods

Tissue Culture. All cells were grown under standard tissue culture conditions. LY-R and LY-S cells were grown in the Fischer’s medium, CB17 in Waymouth’s medium, and 3T3 in DMEM. All media were supplemented with FCS (10%) and antibiotics. Mouse splenocytes were obtained from BALB/c and SWR mice, and mitogen-stimulated (2 µg/ml −1 concanavalin A and 25 µg/ml lipopolysacchaide) cultures in RPMI 1640/20% FCS were set up as described (16). Human lymphocytes were stimulated with phytohemagglutinin and grown for 72 h in RPMI 1640.

Chromosome Radiosensitivity Tests. Four different tests were used: (a) chromosome painting for scoring translocations; (b) classical cytogenetic analysis for scoring dicentric chromosomes; (c) the MN test; and (d) the G2 assay for scoring chromatid breaks and gaps induced in the G2 phase of the cell cycle. For the first three tests, dose-response relationships were established using doses of 1.0, 2.0, and 4.0 Gy of γ rays. For the G2 assay, doses varying from 0.2 Gy to 1.0 Gy were used, depending upon the cell type used. Chromosome painting was performed according to the instructions supplied by the manufacturer of mouse chromosome paints, Cambio. A Leica fluorescence microscope was used to observe painted chromosomes. Classical cytogenetic analysis involved the staining of metaphase figures with Giemsa and scoring dicentric chromosomes using light microscopy. Similarly, for the G2 assay, chromosomes were stained with Giemsa and observed by using light microscopy. The MN test was performed according to the original protocol (17), and micronuclei were scored using light microscopy. Irradiation was carried out either with a 137Cs irradiator (CIS International) for the G2 assay on human lymphocytes, or a 60Co source for the tests involving mouse cells. Dose rates were 4.0 Gy/min and 0.25 Gy/min, respectively.

Q-FISH and Telomerase Detection. Metaphase spreads were hybridized with the PNA telomeric oligonucleotide (CCCTTA), labeled with Cy3 (PE Biosystems) as described (18). Digital images were acquired using a Leica fluorescence microscope coupled with a Photometrics cooled CCD camera and Smart Capture software (Vysis). Telomere fluorescence intensity was analyzed using TFL-Telo software provided by Dr. Peter Lansdorp and Dr. Steven S. S. Poon (Terry Fox Laboratory, Vancouver, British Columbia, Canada).
LY-S is statistically significant (test; LY-S cells determined by Q-FISH. The difference in telomere length between LY-R and IC control (Fig. 1B) for PCR efficiency was included. An internal control (IC) for PCR efficiency was included.

Telomere fluorescence intensity was expressed in TFUs according to a procedure described previously (18). One TFU corresponds to 1 kb of telomere length (18). At least 15 metaphase cells/sample were analyzed in Q-FISH experiments. Telomerase activity was measured using the telomeric repeat amplification protocol assay.

Results

Chromosomal Radiosensitivity and Telomere Length in Mouse Lymphoma Cells. The mouse lymphoma LY-S cell strain was the first reported mammalian strain exhibiting a marked sensitivity to IR (19). This strain was isolated from the parental radio-resistant lymphoma strain, LY-R, after spontaneous transformation in vitro (19). The radiosensitivity of LY-S cells results from a deficiency in DSB repair (20). However, the major mammalian DSB repair protein, DNA-PK, is functional in both strains (20), and the exact nature of DSB repair deficiency in LY-S cells remains unknown.

The aim of this study was to explore the relationship between cellular response to IR and telomere length. To monitor cellular response to IR, four chromosomal radiosensitivity tests were used (see “Materials and Methods”). In agreement with results published previously (21), all tests confirmed that LY-S cells are significantly more radiosensitive than parental LY-R cells (not shown).

To analyze telomere length in these cells, Q-FISH (10, 18) was used because conventional telomere measurements by Southern analysis may not be reliable in mouse cells (22). The average telomere length in DBA mice from which LY-R cells had been isolated is ~40 kb (18). Telomeres in parental radio-resistant LY-R cells were 48 kb long (Fig. 1A and C). In contrast, telomeres in radiosensitive LY-S cells were severely shortened, exhibiting an average length of 7.1 kb (Fig. 1, B and C). Some chromosomes in LY-S cells lacked telomeric signals (see below), suggesting that telomeric sequences have been completely lost or are below the resolution of Q-FISH, i.e., 200 bp.

The analysis of telomerase activity in LY-S and LY-R cells indicated that both strains express telomerase at similar levels (Fig. 1D). Analysis of the telomerase catalytic subunit and telomerase RNA component expression also failed to reveal differences between the strains (not shown). This suggests that the difference in average telomere lengths between cell strains is not attributable to differences in telomerase activity. Monitoring of telomere length in both LY-S and LY-R cells for ~50 mean population doublings revealed that the average telomere length (Fig. 1C) remains stable (not shown). The origin of the shortened telomeres in LY-S cells is unknown and is currently under investigation.

Telomere Length and Chromosomal Radiosensitivity in Lymphocytes from Breast Cancer Patients. The above results, together with observations of shortened telomeres in ska80 mutants, human AT cells, PARP-deficient mice, or C. elegans mrt2 mutant (see “Introduction”), suggest that short telomeres may be associated with cellular and/or chromosomal radiosensitivity. To investigate this possibility further, chromosomal radiosensitivity was assessed in lymphocytes from 24 breast cancer patients and 5 normal donors using the...
G₂ assay. Lymphocytes were exposed to 0.4 Gy γ rays, and frequencies of chromatid breaks and gaps, generated in the G₂ phase of the cell cycle, were determined. Telomere length was measured by Q-FISH in control nonirradiated samples from the same donors. The data (Fig. 2) show an inverse correlation between telomere length and chromosomal radiosensitivity in this group of patients. The correlation is statistically significant (r = 0.503; see Fig. 2 legend). A similar correlation has also been observed between telomere length and patient age. However, this correlation is weaker than the correlation between telomere length and chromosomal radiosensitivity. There was no correlation between radiosensitivity and donor age (not shown).

Selecting Normal Mouse Cells with Different Telomere Lengths. To investigate whether short telomeres in themselves confer chromosomal radiosensitivity, mouse cell lines that show short telomeres as the only obvious defect were selected together with primary cells from corresponding genetic backgrounds as controls. An alternative approach, i.e., initiating primary somatic cell culture, propagating it until telomeres become critically short, and comparing the effects in primary versus senescent cells, was not practical because of ultra-long telomeres and the presence of telomerase activity in mouse cells (22). Furthermore, the range of changes associated with cellular senescence would have made interpretation difficult.

An earlier study has revealed shortened telomeres in the CB17 cell line (10). The CB17 inbred strain from which this cell line originated is essentially BALB/c in its characteristics and genetic composition, although a small element of C57BL is present in the strain’s genome (23). This cell line expresses telomerase and has no obvious hypotopic defect. FISH revealed that telomere length in this cell line is, on average, 11 kb (Fig. 3A). Some chromosomes in CB17 cells lacked telomeric signals completely (Fig. 3B) and were involved in TF (10). The proportion of chromosomes lacking telomeric signals was comparable with that in LY-S cells (Fig. 3B). BALB/c mouse splenocytes were selected as corresponding primary control cells because of their suitability for obtaining the metaphase spreads required for Q-FISH as well as subsequent radiation experiments that require short term in vitro culture (see “Materials and Methods”). The analysis of telomere length in primary splenocytes obtained from a BALB/c mouse revealed a normal telomere length of ~50 kb (Fig. 3A). As expected, all chromosomes in BALB/c splenocytes showed strong telomeric signals (Fig. 3B) and lacked TFs (not shown).

The mouse 3T3 cell line (derived from a Swiss mouse) shows shortened telomeres that are 7 kb on average (Fig. 3A). These cells also show TF because of a complete loss of telomeres in some chromosomes (Fig. 3B). Splenocytes from SWR, an inbred Swiss mouse strain, were used as a control for 3T3. The average telomere length in splenocytes obtained from a SWR mouse was 80 kb (Fig. 3A). All chromosomes in SWR splenocytes showed strong telomeric signals (Fig. 3B). The difference between telomere lengths in cell lines and corresponding primary splenocytes was statistically significant in all cases (t test; P < 0.0001).

Chromosomal Radiosensitivity of Mouse Cells with Differing Telomere Length. The response to IR was monitored in the above cell lines and in corresponding primary splenocytes using four chromosome radiosensitivity tests (Fig. 4). None of the tests revealed statistically significant differences in response to IR between mouse cells with short telomeres and corresponding genetically normal cells (Fig. 4). In some cases, frequencies of chromosome damage were slightly higher in cells with longer telomeres (Fig. 4C). Comparison of sensitivity to IR in BALB/c and SWR cells did not reveal any statistically significant difference either (Fig. 4), although these two types of mice are genetically different and have different telomere lengths (Fig. 3A). Therefore, these results suggest that short telomeres and chromosomal radiosensitivity are not always associated.

Discussion

Many radiosensitive eukaryotic cells show defects in telomere maintenance. Most defects involve severe telomere shortening as

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Fig. 4. Chromosome radiosensitivity in mouse cell lines and corresponding primary cells from the same genetic backgrounds. A, frequencies of dicentric (Dic) chromosomes. B, frequencies of translocations (T) involving chromosomes 2 and 11, identifiable with chromosome painting. C, G₂ assay. Note that in the G₂ assay, the frequency of chromatid breaks and gaps (B/G) declines with time after irradiation (dose used was 1.0 Gy). D, MN test. In all cases, at least two independent experiments were performed. In each experiment, at least 100 cells/point were analyzed. Results presented are the means from two experiments and are expressed on a per-cell basis. Statistical analysis did not reveal significant differences in response to IR between different cell types.

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4 J. McIlrath and P. Slijepcevic, unpublished observations.
5 P. Slijepcevic, unpublished results.
exemplified by the yku70-deficient yeast (2), the C. elegans mrt2 mutant (15), human AT cells (6), and PARP-deficient mice (14). The analysis of radiosensitive LY-S mouse lymphoma cells presented here revealed a 7-fold reduction in telomere length as compared with parental cells (Fig. 1). LY-S cells have a defect in DSB repair (21) despite functional DNA-PKcs. Although telomerase activity is normal in both LY-R and LY-S cells (Fig. 1D), telomere length in LY-S cells (Fig. 1C) is comparable with that of late-generation telomerase-null mice (22), whereas parental LY-R cells have normal telomere length (Fig. 1C). Telomere length is maintained at these levels in both LY-S and parental LY-R cells for at least 50 mean population doublings (see “Results”), suggesting that gradual telomere shortening may not be responsible for the loss of telomeric sequences in LY-S cells. It seems more likely that a fast and severe telomere shortening may correlate with the transformation of the radiosensitive LY-R phenotype into the radiosensitive LY-S phenotype. Observation of accelerated telomere shortening in radiosensitive AT cells (6) and the finding that other radiosensitive eukaryotic cells show severely shortened telomeres (2, 14, 15) support this possibility.

On the basis of these observations, it was reasoned that telomere length might be used as a marker for chromosomal radiosensitivity. An inverse significant correlation between telomere length and chromosomal radiosensitivity in lymphocytes from breast cancer patients (Fig. 2) seems to support this hypothesis. Taken together, these data suggest that telomere length may be used as a potential diagnostic marker for detecting chromosomal radiosensitivity. There is a good correlation between G2 chromosomal radiosensitivity and predisposition to breast cancer (24). It will be of interest to analyze telomere length and telomere maintenance mechanisms in a larger number of breast cancer cases and in other types of cancer with the purpose of testing the above hypothesis more rigorously.

However, the analysis of mouse cell lines with short telomeres as the only obvious defect failed to confirm the correlation between telomere length and chromosomal radiosensitivity. Thus, short telomeres in themselves may not necessarily confer chromosomal radiosensitivity in vitro, at least in the case of acute γ-ray exposure. Alternatively, telomeres in CB17 and 3T3 cells may not have been sufficiently shortened to significantly affect cellular response to IR. However, judging by similar frequencies of chromosomes without telomeres in LY-S, CB17, and 3T3 cells and subsequent formation of TF, indicative of the impairment of telomere function, this seems unlikely (Fig. 2). Also, it is possible that in established cell lines, mechanisms that confer radiosensitivity may be different from those in normal primary cells. For example, severe telomere shortening causes a p53-mediated apoptotic response (25). Established mouse cell lines usually lose either p53 or p19ARF (26), and this could affect cellular radiation response by tolerating short telomeres. In addition, it should be noted that cell type (spleenocyte/fibroblast) also may be influencing chromosomal radiosensitivity.

In conclusion, the observations presented here suggest that telomere length and chromosomal radiosensitivity are inversely correlated in some cases. This may reflect common pathways in DNA metabolism that affect both phenotypes. Additional research is required to exploit the potential clinical application of our finding.

Note Added in Proof


References

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