Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 367 (2008) 84–89
BBRC
www.elsevier.com/locate/ybbrc

Telomere length inversely correlates with radiosensitivity in human carcinoma cells with the same tissue background

Ya-Hua Zhong [1], Zheng-Kai Liao [1], Fu-Xiang Zhou, Cong-Hua Xie, Chuang-Ying Xiao, Dong-Feng Pan, Zhi-Guo Luo, Shi-Quan Liu, Yun-Feng Zhou *

Department of Chemo-Radiotherapy Oncology, Zhongnan Hospital, Wuhan University, Wuhan, Hubei 430071, China

Received 2 December 2007
Available online 26 December 2007

Abstract

A relationship between telomeres and radiosensitivity has been established by several studies based on non-mammalian model systems, mouse models, and few human genetic diseases. However, the relationship has not been proven in human carcinoma cells, which have more clinical significance than these other models. The present study aims to determine whether telomere length is related to radiosensitivity in human carcinoma cells, and to examine the influence of tissue or genetic background. Two HEp-2 larynx squamous carcinoma cell lines, eight hepatocellular carcinoma cell lines, and five breast cancer cell lines were used. Telomere length was determined by terminal restriction fragment (TRF) Southern blot analysis and cell survival was measured by a colony-forming assay. Our results indicated that there was a significant negative correlation of telomere length and radiosensitivity in the same tissue-derived cell lines, with or without the same genetic background. Thus, telomere length may be used as a promising tool to predict the radiosensitivity of human carcinomas.

Keywords: Telomere; Radiosensitivity; Carcinoma; Humans

Telomeres are the physical ends of eukaryotic chromosomes, composed of a variable number of tandem repeats of DNA that are made up of (TTAGGG)n repeats [1]. Each round of DNA replication is accompanied by telomere shortening, due to the incomplete replication of DNA ends. In most normal human cells, the function of telomeres appears to be a molecular counting device that registers cell divisions and triggers a proliferative arrest when telomeres erode to specific lengths [2]. In contrast, telomere length is stable in immortalized cells and cancer cells. To date, the molecular regulation of telomere length has not been well elucidated.

Radiation has been a well established modality for treating more than 70% of patients with cancer. Since the radiosensitivity of tumor cells is still unclear, the prognosis of patients with carcinoma who received radiotherapy is unsatisfactory. From this point of view, prediction of radiosensitivity could be of great value in the treatment of malignant tumors.

A link between radiosensitivity and telomere maintenance is detectable in yeast and *Caenorhabditis elegans*, as several proteins are involved both in telomere length regulation and radiosensitivity [3,4]. More recently, studies in mice have provided a mammalian precedent. Several mouse models, including mice deficient in Ku, DNA-PKcs (Prkdc), Parp, and Atm, all of which are radiosensitive in vivo, also show clear telomere alterations [5]. In humans, the evidence that telomere shortening might lead to radiosensitivity is far less direct, although human genetic diseases characterized by clinical radiosensitivity, such as ataxia-telangiectasia (A-T) and Nijmegen breakage syndrome (NBS), show alterations

Abbreviations: TRF, terminal restriction fragments; SF2, surviving fraction at 2 Gy; DSBs, DNA double strand breaks.

* Corresponding author. Fax: +86 27 67812889.
E-mail address: yfzhouwhu@gmail.com (Y.-F. Zhou).

[1] These authors contributed equally to this study.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.12.078

in telomere maintenance [6]. Moreover, an inverse correlation between telomere length and chromosome radiosensitivity was observed in murine lymphoma cells (L5178Y-S and L5178Y cells) and lymphocytes from 24 breast cancer patients and five normal individuals [7].

Nevertheless, an association between telomere length and radiosensitivity has not been established yet in human carcinoma cells, whose radiosensitivity determines the therapeutic effect of radiotherapy. Furthermore, it is not clear how tissue origin influences this association in human carcinoma cells. Therefore, the aim of this study was to investigate whether tissue or genetic differences have an impact on the relationship between telomere length and radiosensitivity in 15 human carcinoma cell lines from various tissues. This model system, which affords a comparison of cells possessing similar genetic backgrounds, the same tissue background with different genetic backgrounds, and different tissue backgrounds, is particularly relevant to reduce influencing factors and enhance comparability.

To our knowledge, systematic in vitro studies, aimed at testing the relationship between telomere length, radiosensitivity, and influence of tissue origin in human carcinoma cells have not been published.

Materials and methods

Cell culture. Human HEp-2 larynx squamous carcinoma cell line and five breast cancer cell lines (ZR-75-30, MCF-7, MDA-MB-435S, T47D, 1590) were obtained from the China Center for Type Culture Collection (CCTCC). Eight cell strains (MHCC97-L, HMCC97-H, HCCLM1, HCCLM2, HCCLM3, HCCLM4, HCCLM6, and HCCLM7) from human hepatocellular carcinoma cell line MHCC97 were kindly provided by Dr. Li (Department of Oncology, Zhongnan Hospital, Wuhan University, Wuhan, China) [8–10]. All cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplement with 10% foetal calf serum (Hyclone, Logan, UT) at 37 °C in humidified atmosphere containing 5% CO_2.

Isolation of radioresistant variant cell line. The radioresistant human larynx squamous carcinoma cell line, HEp-2R, was isolated by repeated radiation exposure as described [11]. Briefly, exponentially growing HEp-2 cells in 50 cm^2 culture flasks were exposed to a dose of 637 cGy ^{60}Co γ-rays (20 × 20 cm, 80 cm, 61.3 cGy/min) at room temperature, and the culture medium was changed after radiation. When the irradiated cells reached the end of the exponential growth phase, they were trypsinized and appropriate numbers of cells were plated in 50 cm^2 culture flasks. Re-irradiation was performed 1 day after seeding. Designated HEp-2R, this cell line was isolated by repeating this procedure 12 times, and has already been maintained for >50 passages.

Measurement of telomere length. Mean telomere length was determined by TRF analysis using the Telo TAGGG Telomere Length Assay Kit (Roche, Basel, Switzerland). According to the manufacturer's instructions, isolated DNA (4 μg) was digested using a HinfI/RsaI enzyme mixture for 2 h at 37 °C. Digested DNA samples were then resolved by 0.7% agarose gel electrophoresis for 2–4 h at 5 V/cm. Gels were denatured, neutralized, and transferred to a positively-charged nylon membrane which was then hybridized with a biotinylated telomere probe. Mean TRF lengths were analyzed from a densitometric scan of the autoradiogram by the Bio-ID gel photograph system (VL Co., France) and calculated using the formula $TRF = \Sigma(OD_i)/\Sigma(OD_i/Li)$, where OD_i is the chemiluminescent signal and L_i is the length of the TRF fragment at position i. The positive control DNA (Control-DNA, low and Control-DNA, high) supplied with the kit were purified genomic DNA from immortal cell lines. The mean TRF length of these cell lines has been determined to be 3.9 and 10.2 kb, respectively.

Colony-forming assay. Cells were trypsinized at 37 °C for 5–10 min, and pipetted eight times to keep the clumped cells as a single cell suspension. The single cell suspension was adjusted and seeded into 25 cm^2 flasks at various densities based on our results of pre-experiments. Then, cells were left to settle over night, and exposed to irradiation at room temperature, followed by immediate incubation at 37 °C, 5% CO_2 for 14–20 days. After fixation and staining with 1% w/v crystal violet (Sigma, St. Louis, MO) in dehydrated alcohol, colonies of >50 cells were scored. Surviving fractions were evaluated relative to 0 Gy radiation treated controls.

Statistical analysis. All results were expressed as means ± SD. Significance tests were carried out on the date groups by independent sample *t*-test. Differences between the survival fractions of groups were analyzed by chi-squared test. The relationship between two variables was assessed by linear regression analysis. Values of $P < 0.05$ were considered significant.

Results

Isolation of radioresistant HEp-2R human larynx squamous carcinoma cell line

A radioresistant variant was isolated from the radiation-sensitive HEp-2 human larynx squamous carcinoma cell line as described [11]. The isolation was performed in the HEp-2 cells using repetitive exposures 637 cGy radiation. In order to test the stability of HEp-2R cells, evaluated its surviving fraction in 2 and 4 Gy radiation every 4 weeks during subculture. The results were compared with those of untreated HEp-2R cells frozen for 4 months, and no significant differences were found between them. We did find differences between Hep-2R and Hep-2 cells, including morphology, chromosomes, cell growth kinetics, cell distribution and gene expression. These findings suggested that a radioresistant variant cell line, HEp-2R, was isolated [12].

Telomere length and radiosensitivity in larynx squamous carcinoma cell lines with similar genetic backgrounds

The telomere lengths of HEp-2 and HEp-2R cell lines were determined by restriction fragment analysis. We found that the HEp-2 and HEp-2R cell lines had an average telomere length of about 3.76 ± 0.015 and 11.12 ± 0.005 kb ($P < 0.01$), respectively (Fig. 1A). The survival curves were analyzed according to the multi-target single-hit (SHMT) model ($S = 1\text{-}(1\text{-}\exp(\text{-}D/D_0))N$), normalizing for plating efficiency of mock-irradiated cells (Fig. 1B). The extrapolation numbers were $D_0 = 3.24$ Gy, $D_q = 1.90$ Gy, and $N = 1.80$ for HEp-2R cells, and $D_0 = 2.06$ Gy, $D_q = 1.01$ Gy, and $N = 1.64$ for HEp-2 cells. The surviving fractions at 2 Gy (SF2) for the HEp-2R cells (0.680 ± 0.031) was significantly greater than that of the HEp-2 cells (0.415 ± 0.027) (SF2 $\chi 2 = 63.96$, $P < 0.01$). Hence, the average telomere length in the radioresistant HEp-2R line was greater than that in the radiosensitive HEp-2 cell line.

Telomere length and radiosensitivity in hepatocellular carcinoma cell lines with similar genetic backgrounds

Using a metastatic human hepatocellular carcinoma (HCC) cell line, MHCC97 [13], as the parental cells, Li

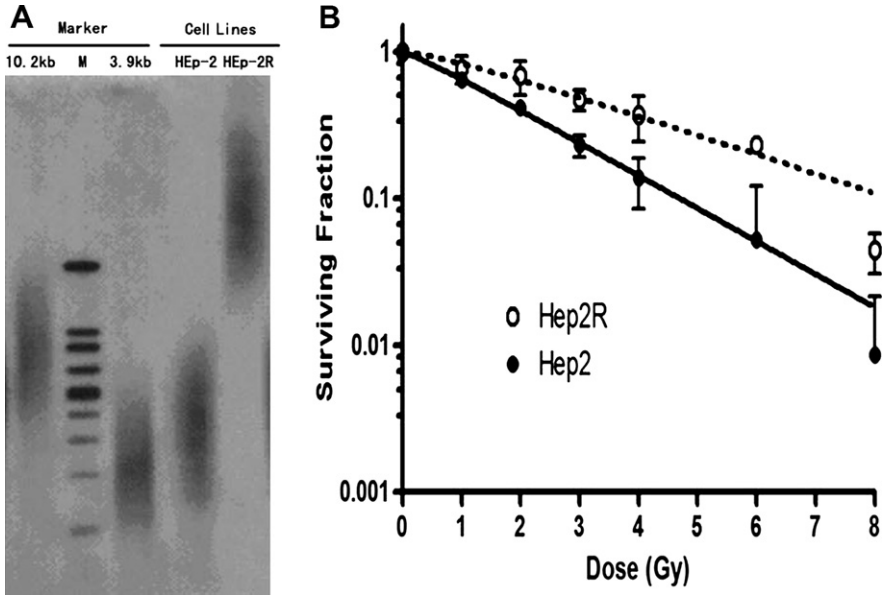

Fig. 1. Telomere length and radiosensitivity in HEp-2 and HEp-2R cells. (A) Representative average telomere length in HEp-2 and HEp-2R cells determined by restriction fragment analysis. (B) The clonogenic survival curves of HEp-2 and HEp-2R cells. The survival curves were analyzed according to the SHMT model.

et al. subsequently established eight cell lines (MHCC97-L, HMCC97-H, HCCLM1, HCCLM2, HCCLM3, HCCLM4, HCCLM6, and HCCLM7) with different spontaneous metastatic potential [8–10]. These cell lines from one origin were grouped together because they have a similar genetic background and could be used as a human cell-culture model to directly investigate both radiosensitivity and telomeres. TRF length determined by Southern blot analysis was different among these cell lines ($P < 0.05$), supporting the idea of the genetic background difference (Fig. 2A).

SF2 was used as an index of clonogenic cellular radiosensitivity [14,15]. Fig. 2B shows the correlation between radiosensitivity and TRF in hepatocellular carcinoma cell lines. Linear regression analysis was used to establish a determination coefficient (r^2) of 0.8737 for the relationship between TRF length and radiosensitivity (SF2) ($P < 0.05$). It was obvious that different radiosensitivities between cell lines depended on the length of the telomere. To surmise, telomeres in radiosensitive cell lines were shorter than that in radioresistant cell lines.

Telomere length and radiosensitivity in breast cancer cell lines with different genetic backgrounds and the same tissue background

Five cell lines, ZR-75-30, MCF-7, MDA-MB-435S, T47D, and 1590, came from mammary glands of different individuals. The general findings of TRF length in these cell lines are shown in Fig. 3A. It was found that for telomere lengths, MCF-7>T47D>1590>MDA-MB-435S>ZR-75-30, and these differences were significantly significant ($P < 0.05$). TRF length was negatively correlated with radiosensitivity ($r^2 = 0.9113$, $P < 0.05$, Fig. 3B). These result indicated there was a significant inverse correlation between tissue-adjusted telomere length and radiosensitivity.

Telomere length and radiosensitivity in human carcinoma cell lines with different tissue backgrounds

The correlation between radiosensitivity and TRF length in different carcinoma cell lines is shown (Fig. 4). The tissues chosen in this study were selected in order to represent different tissue types. In particular, breast cancer, hepatocellular carcinoma, and larynx squamous cell carcinoma may be important cell lines to study in terms of telomere maintenance and radiosensitivity in human carcinomas. However, we did not find a correlation between TRF length and radiosensitivity ($r^2 = 0.1793$, $P > 0.05$). In this case, telomere-related radiosensitivity reflected differences in cell growth in the tissue types examined.

Discussion

Some studies have reported that there is a relationship between telomere length and radiosensitivity. However, no detailed study has been published dealing with this relationship in human carcinoma cells. Because of its clinical potential, we focused on investigating the relationship between telomere length and radiosensitivity. Analysis of the origin of radiosensitivity in fifteen human carcinoma cell lines provided new insights into the nature of telomere maintenance. Our results indicated that telomere length inversely correlated with radiosensitivity in human carcinoma cells. Furthermore, this relationship was dependent on not only genetic background but also tissue background.

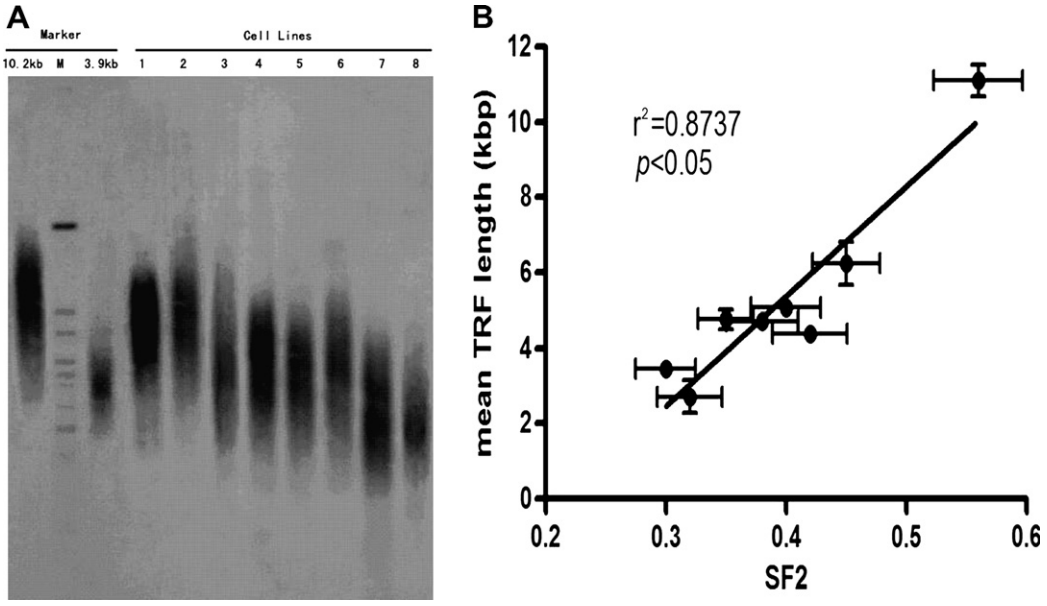

Fig. 2. (A) Representative average telomere length in hepatocellular carcinoma cells determined by restriction fragment analysis. 1: HMCC97-H; 2: MHCC97-L; 3: HCCLM2; 4: HCCLM3; 5: HCCLM4; 6: HCCLM6; 7: HCCLM7; 8: HCCLM1. (B) Example of correlations between radiosensitivity (SF2) and the TRF length in hepatocellular carcinoma cell lines.

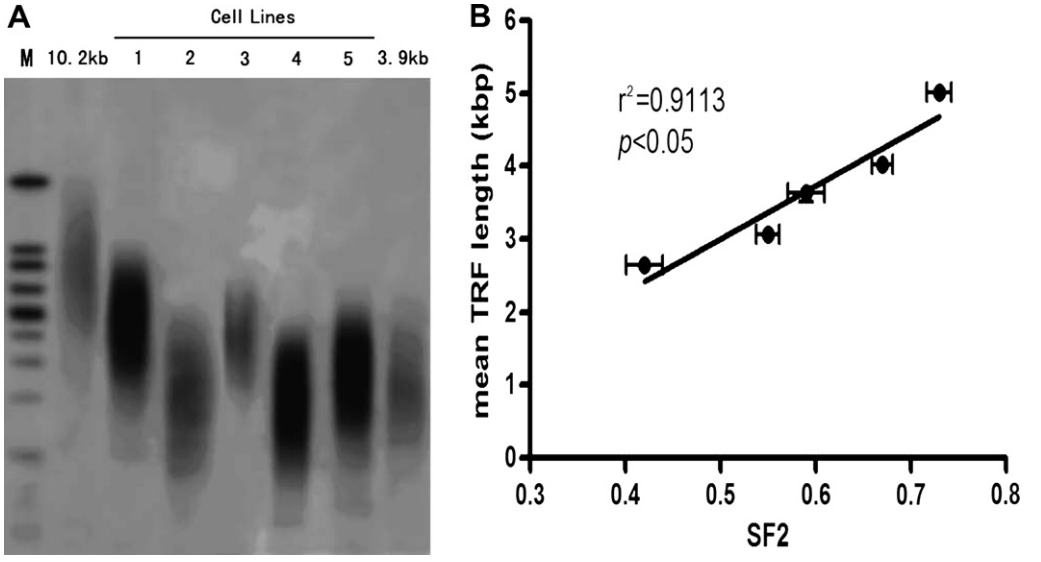

Fig. 3. (A) Representative average telomere length in breast cancer cell lines determined by restriction fragment analysis. 1: MCF-7; 2: MDA-MB-435S; 3: T47D; 4: ZR-75-30; 5: 1590. (B) Example of correlations between radiosensitivity (SF2) and the TRF length in breast cancer cells.

Ionizing radiation increases DNA double strand breaks (DSBs), which is probably the most dangerous type of DNA damage that exists within the cell [16]. Irradiation damage to DNA triggers the DNA repair procedure in cells, and chromosomal stability and cellular DNA repair ability determine the termination of these processes [edit okay?], reflecting radiosensitivity [17]. Although telomere length regulation in mammalian cells is complex, there is increasing evidence that telomere maintenance and DNA stabilization mechanisms are closely related. These studies suggest a telomere-dependent role for radiosensitivity. The reasons for this correlation are discussed below.

Firstly, telomeres serve to protect natural DNA ends from being recognized as double-stranded breaks that would otherwise activate DNA damage checkpoint responses or participate in recombination events [18]. In most normal human cells, telomeric DNA is progressively lost with each round of cell division. Eventually, telomeres are shortened to a critical limit and lose their ability to protect chromosome ends. Consequently, widespread chromosomal aberrations such as end-to-end fusion, aneuploidy and degradation occur, leading to replicative senescence triggering cell death through apoptosis [19]. A stable telomere length is required at the chromosome ends of tumor

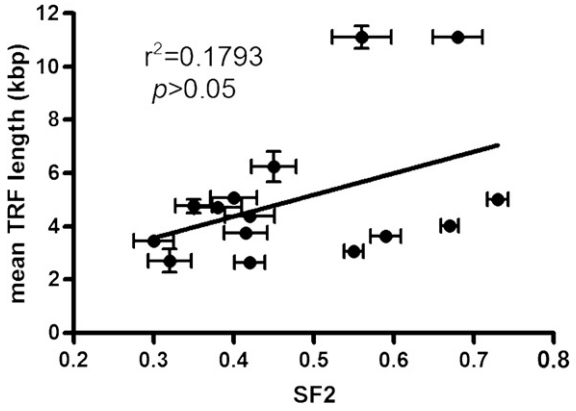

Fig. 4. Example of correlations between radiosensitivity (SF2) and the terminal restriction fragment (TRF) length in fifteen human carcinoma cell lines.

cells to maintain chromosome stability and prevent chromosome fusions.

In addition, since the synthesis and maintenance of telomeres is mainly mediated by telomerase, telomere length can reflect the DNA repair ability of telomerase. Telomerase is a ribonucleoprotein complex that includes an RNA template and a reverse transcriptase catalytic subunit [20]. Low or undetectable levels of telomerase activity were found in most normal somatic cells in humans. About 85% of human tumors possess telomerase activity, whereas the remainder maintain telomeres via a recombination-mediated process termed alternative lengthening of telomeres (ALT) [21], suggesting that telomerase is the dominant pathway to maintain telomere length. The telomere participates in processes of chromosomal repair, as evidenced by the synthesis of telomere repeats at DSBs [22,23]. We have previously shown that telomerase inhibitor Zidovudine (AZT) could depress the activity of telomerase, shorten telomere length and increase radiosensitivity in the human malignant glioma cell line U251 [24]. Irradiation could upregulate telomerase activity in HEp-2 cells, which may be a reaction to DNA damage [25]. It has been suggested that a telomerase-dependent telomere maintenance mechanism might be important in DNA repair, as is the case in the rat mammary epithelium [26].

Furthermore, telomeres might serve as storage sites for essential components of the DNA repair machinery [27]. Several studies demonstrate that the DNA repair proteins such as yKu80 and Sir3p, which are found in telomeres, are released from telomeres to DNA double-strand breaks [28,29]. Ku proteins can bind to telomeric sequences and prevent end-to-end fusions [30]. Thus, telomere length reflecting the capacity of the telomere might relate to the DNA repair ability.

Taken together, the findings presented here suggest that telomere length may be one of the important ingredients of chromosomal radiosensitivity in human carcinoma cells. Further investigation will be needed to clarify the telomere maintenance mechanisms in detail.

The length and maintenance of telomeres differ among species [31]. Although mice provide a powerful tool for understanding cancer progression, there are significant differences between human and mouse telomere biology. Telomere length in humans is much shorter than that in Mus musculus, a common laboratory species [32]. In contrast to human tissues, many somatic mouse tissues have significantly active telomerase [33], and telomere shortening and telomerase activation have little effect on mouse tumorigenesis [34]. Thus, telomere signalling pathways may be differentially regulated in human and mouse [35]. In spite of these differences, telomere shortening has been extensively studied in mice, especially in telomerase-deficient knockout mice [36]. Since some of the most critical pathological processes associated with human aging are not well recapitulated in mouse, the mouse model of telomere shortening may be of limited usefulness in understanding the role of telomere length or telomerase activity [32]. There is a need for an experimental animal which more closely mimics the human situation in order to study the role of telomere shortening in tissue and organism senescence [31]. Therefore, the data derived from studies of mouse telomere biology need to be interpreted very carefully.

In human, several autosomal genetic diseases such as Nijmegen breakage syndrome (NBS) and ataxia-telangiectasia (A-T) display many abnormalities, including telomere shortening and sensitivity to ionizing radiation [6]. McIlrath et al. have shown that telomere length and chromosomal radiosensitivity are inversely correlated in lymphocytes from 24 breast cancer patients and five normal individuals [7]. Nevertheless, these findings may not directly extrapolate to comparable aspects of cancer biology, since these cells significantly differ from carcinoma cells both in genotype and phenotype. As mentioned previously, the development of various tumor cell lines allows the direct investigation of both radiosensitivity and telomeres. Our results presented here suggest that telomere length may be used as a potential diagnostic marker for estimation of radiosensitivity in human carcinoma cells, at least in breast cancer, hepatocellular carcinoma, and larynx squamous cell carcinoma. Although we do not have a clear explanation for this discrepancy, the radiosensitivity may vary among cell types or genetic backgrounds. The present study provided the first evidence that telomere length of cells with same tissue and genetic background was maintained independently of other intracellular factors.

In conclusion, we report in this study that telomere may be used as a predictor of radiosensitivity. By extension, these data provide a novel mechanism for tissue-related telomere maintenance differences and suggest tissue-specific regulation of telomeres during the development and DNA repairing in the human carcinomas. Telomere maintenance mechanisms therefore appear to be a promising target for radiosensitization. Elucidation of the molecular mechanisms may improve therapeutic regulation of radiotherapy in human tumor cells.

Acknowledgment

This work was supported by the Natural Science Foundation of China (30171063).

References

[1] J. Meyne, R.L. Ratliff, R.K. Moyzis, Conservation of the human telomere sequence (TTAGGG)n among vertebrates, Proc. Natl. Acad. Sci. USA 86 (1989) 7049–7753.
[2] W.E. Wright, J.W. Shay, Historical claims and current interpretations of replicative aging, Nat. Biotechnol. 20 (2002) 682–688.
[3] Y. Ono, K. Tomita, A. Matsuura, T. Nakagawa, H. Masukata, M. Uritani, T. Ushimaru, M. Ueno, A novel allele of fission yeast rad11 that causes defects in DNA repair and telomere length regulation, Nucleic Acids Res. 31 (2003) 7141–7149.
[4] S. Ahmed, J. Hodgkin, MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*, Nature 403 (2000) 159–164.
[5] P. Slijepcevic, Is there a link between telomere maintenance and radiosensitivity? Radiat. Res. 161 (2004) 82–86.
[6] E. Cabuy, C. Newton, G. Joksic, L. Woodbine, B. Koller, P.A. Jeggo, P. Slijepcevic, Accelerated telomere shortening and telomere abnormalities in radiosensitive cell lines, Radiat. Res. 164 (2005) 53–62.
[7] J. McIlrath, S.D. Bouffler, E. Samper, A. Cuthbert, A. Wojcik, I. Szumiel, P.E. Bryant, A.C. Riches, A. Thompson, M.A. Blasco, R.F. Newbold, P. Slijepcevic, Telomere length abnormalities in mammalian radiosensitive cells, Cancer Res. 61 (2001) 912–915.
[8] Y. Li, Z.Y. Tang, S.L. Ye, Y.K. Liu, J. Chen, Q. Xue, J. Chen, D.M. Gao, W.H. Bao, Establishment of cell clones with different metastatic potential from the metastatic hepatocellular carcinoma cell line MHCC97, World J. Gastroenterol. 7 (2001) 630–636.
[9] Y. Li, Y. Tang, L. Ye, B. Liu, K. Liu, J. Chen, Q. Xue, Establishment of a hepatocellular carcinoma cell line with unique metastatic characteristics through in vivo selection and screening for metastasis-related genes through cDNA microarray, J. Cancer Res. Clin. Oncol. 129 (2003) 43–51.
[10] Y. Li, B. Tian, J. Yang, L. Zhao, X. Wu, S.L. Ye, Y.K. Liu, Z.Y. Tang, Stepwise metastatic human hepatocellular carcinoma cell model system with multiple metastatic potentials established through consecutive in vivo selection and studies on metastatic characteristics, J. Cancer Res. Clin. Oncol. 130 (2004) 460–468.
[11] N. Mitsuhashi, T. Takahashi, H. Sakurai, M. Nozaki, T. Akimoto, M. Hasegawa, Y. Saito, H. Matsumoto, K. Higuchi, K. Maebayashi, H. Niibe, A radioresistant variant cell line, NMT-1R, isolated from a radiosensitive rat yolk sac tumour cell line, NMT-1: differences of early radiation-induced morphological changes, especially apoptosis, Int. J. Radiat. Biol. 69 (1996) 329–336.
[12] F.X. Zhou, J. Xiong, Z.G. Luo, Z.K. Liao, S.Q. Liu, and Y.F. Zhou, Human radiosensitive-radioresistant cell lines model and its cdna expression profiles, in preparation).
[13] J. Tian, Z.Y. Tang, S.L. Ye, Y.K. Liu, Z.Y. Lin, J. Chen, Q. Xue, New human hepatocellular carcinoma (HCC) cell line with highly metastatic potential (MHCC97) and its expressions of the factors associated with metastasis, Br. J. Cancer 81 (1999) 814–821.
[14] S. Wada, T. Van Khoa, Y. Kobayashi, T. Funayama, K. Ogihara, S. Ueno, N. Ito, Prediction of cellular radiosensitivity from DNA damage induced by gamma-rays and carbon ion irradiation in canine tumor cells, J. Vet. Med. Sci. 67 (2005) 1089–1095.
[15] T. Bjork-Eriksson, C. West, E. Karlsson, C. Mercke, Tumor radiosensitivity (SF2) is a prognostic factor for local control in head and neck cancers, Int. J. Radiat. Oncol. Biol. Phys. 46 (2000) 13–19.
[16] K.K. Khanna, S.P. Jackson, DNA double-strand breaks: signaling, repair and the cancer connection, Nat. Genet. 27 (2001) 247–254.
[17] R. Kanaar, J.H. Hoeijmakers, Recombination and joining: different means to the same ends, Genes Funct. 1 (1997) 165–174.
[18] F.A. Goytisolo, E. Samper, J. Martin-Caballero, P. Finnon, E. Herrera, J.M. Flores, S.D. Bouffler, M.A. Blasco, Short telomeres result in organismal hypersensitivity to ionizing radiation in mammals, J. Exp. Med. 192 (2000) 1625–1636.
[19] C.E. Jefford, I. Irminger-Finger, Mechanisms of chromosome instability in cancers, Crit. Rev. Oncol. Hematol. 59 (2006) 1–14.
[20] T.R. Cech, Beginning to understand the end of the chromosome, Cell 116 (2004) 273–279.
[21] S. Chang, C.M. Khoo, M.L. Naylor, R.S. Maser, R.A. DePinho, Telomere-based crisis: functional differences between telomerase activation and ALT in tumor progression, Genes Dev. 17 (2003) 88–100.
[22] P.S. Meltzer, X.Y. Guan, J.M. Trent, Telomere capture stabilizes chromosome breakage, Nat. Genet. 4 (1993) 252–255.
[23] J.P. Murnane, L.C. Yu, Acquisition of telomere repeat sequences by transfected DNA integrated at the site of a chromosome break, Mol. Cell Biol. 13 (1993) 977–983.
[24] F.X. Zhou, Z.K. Liao, J. Dai, J. Xiong, C.H. Xie, Z.G. Luo, S.Q. Liu, Y.F. Zhou, Radiosensitization effect of zidovudine on human malignant glioma cells, Biochem. Biophys. Res. Commun. 354 (2007) 351–356.
[25] Z.K. Liao, F.X. Zhou, Z.G. Luo, W.J. Zhang, J. Xiong, J. Bao, G. Han, M.S. Zhang, C.H. Xie, Y.F. Zhou, Radio-activation of hTERT promoter in larynx squamous carcinoma cells: an 'indirected-activator' strategy in radio-gene-therapy, Oncol. Rep. 19 (2008) 281–286.
[26] D. Varon, C. Jiang, C. Hedican, J.S. Dome, C.B. Umbricht, L.A. Carey, H.J. Thompson, S. Sukumar, Telomerase activity in the normal and neoplastic rat mammary gland, Cancer Res. 57 (1997) 5605–5609.
[27] K.K. Wong, S. Chang, S.R. Weiler, S. Ganesan, J. Chaudhuri, C. Zhu, S.E. Artandi, K.L. Rudolph, G.J. Gottlieb, L. Chin, F.W. Alt, R.A. DePinho, Telomere dysfunction impairs DNA repair and enhances sensitivity to ionizing radiation, Nat. Genet. 26 (2000) 85–88.
[28] S.G. Martin, T. Laroche, N. Suka, M. Grunstein, S.M. Gasser, Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast, Cell 97 (1999) 621–633.
[29] K.D. Mills, D.A. Sinclair, L. Guarente, MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks, Cell 97 (1999) 609–620.
[30] E. Samper, F.A. Goytisolo, P. Slijepcevic, P.P. van Buul, M.A. Blasco, Mammalian Ku86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang, EMBO Rep. 1 (2000) 244–252.
[31] H. Cherif, J.L. Tarry, S.E. Ozanne, C.N. Hales, Ageing and telomeres: a study into organ- and gender-specific telomere shortening, Nucleic Acids Res. 31 (2003) 1576–1583.
[32] N.P. Weng, R.J. Hodes, The role of telomerase expression and telomere length maintenance in human and mouse, J. Clin. Immunol. 20 (2000) 257–267.
[33] K.R. Prowse, C.W. Greider, Developmental and tissue-specific regulation of mouse telomerase and telomere length, Proc. Natl. Acad. Sci. USA 92 (1995) 4818–4822.
[34] D. Broccoli, L.A. Godley, L.A. Donehower, H.E. Varmus, T. de Lange, Telomerase activation in mouse mammary tumors: lack of detectable telomere shortening and evidence for regulation of telomerase RNA with cell proliferation, Mol. Cell Biol. 16 (1996) 3765–3772.
[35] E. Marinova, S. Han, B. Zheng, Age-associated deficiency in activation-induced up-regulation of telomerase activity in CD4+ T cells, Clin. Exp. Immunol. 140 (2005) 258–264.
[36] S.E. Artandi, R.A. DePinho, Mice without telomerase: what can they teach us about human cancer? Nat. Med. 6 (2000) 852–855.