Radiation-Induced Changes of Telomerase Activity in a Human Ewing Xenograft Tumor

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Aim: The effect of ionizing irradiation on telomerase activity and further associated biological factors was evaluated in a human Ewing tumor xenograft model on nude mice.
Material and Methods: The human Ewing tumor cell line STA-ET-1 was established in a nude mouse model. Initially, the dose-response relationship for the tumor model was established. For the radiation experiments two dose levels were chosen: 5 Gy and 30 Gy. After 5 Gy, there was no significant growth delay whereas after 30 Gy there was a marked growth delay without the induction of a complete remission. Tumors were examined 6, 12, 24, 48, 72, and 96 hours post irradiation. After irradiation with 30 Gy further time points were 6, 9, 12 and 15 days. For each dose and time group, three tumors were evaluated.
Results: There was a reduction of telomerase activity after 5 Gy to 50% (not statistically significant) after 3 days; however, after 30 Gy there was a reduction of telomerase activity to 23% of the initial value after 6 days ($p = 0.001$). Telomerase activity correlated with the expression of human telomerase reverse transcriptase (hTERT), but not with the expression of telomerase-associated protein (TP1) and human telomerase RNA (hTR). The maximal amounts of necrosis or apoptosis after 30 Gy were 19% and 6,9%, respectively.
Conclusions: Ionizing radiation reduces telomerase activity and the expression of hTERT which cannot be explained by the induction of necrosis or apoptosis alone. The reduction of telomerase activity may contribute to delayed cell death after radiotherapy. The combined use of radiation and specific telomerase inhibitors may be a potentially synergistic treatment strategy.

Key Words: Ewing tumor · Xenograft · Telomerase · Apoptosis

Strahlenther Onkol 2002;178:701–8
DOI 10.1007/s00066-002-0992-x

Bestrahlungsinduzierte Änderungen der Telomeraseaktivität eines humanen Ewing-Xenografttumors

Ziel: Die Wirkung von ionisierender Strahlung auf die Telomeraseaktivität und weiterer assoziierter biologischer Parameter wurde an einem humanen Ewing-Tumor-Xenograft-Modell auf der Nacktmaus untersucht.
Material und Methode: Die humane Ewing-Tumor-Zelllinie STA-ET-1 wurde auf der Nacktmaus etabliert. Zuerst wurde die Dosis-Wirkungs-Beziehung für den Tumor etabliert. Für die Bestrahlungsexperimente wurden zwei Bestrahlungsdosen gewählt: 5 Gy und 30 Gy. Nach 5 Gy wurde keine Wachstumsverzögerung beobachtet, nach 30 Gy trat eine deutliche Wachstumsverzögerung auf, ohne eine komplette Remission zu induzieren. Die Tumoren wurden 6, 12, 24, 48, 72 und 96 Stunden nach Bestrahlung untersucht. Nach 30 Gy wurden zusätzliche Zeitpunkte nach 6, 9, 12 und 15 Tagen gewählt. Für jede Dosis- und Zeitgruppe wurden drei Tumoren untersucht.
Ergebnisse: Nach 5 Gy kam es zu einer statistisch nicht signifikanten Reduktion der Telomeraseaktivität auf 50% nach 3 Tagen (Abbildung 3a). Nach 30 Gy war die Telomeraseaktivität nach 6 Tagen auf 23% reduziert ($p = 0,001$, Abbildung 4a). Die Telomeraseaktivität korrelierte mit der Expression von hTERT (Human Telomerase Reverse Transcriptase, Abbildungen 3b und 4b), nicht jedoch mit der Expression von TP1 (Telomerase-assoziiertes Protein) und hTR (Human Telomerase RNA). Der maximale Nekrose- und Apoptoseanteil betrug 19% und 6,9% (Abbildung 6).

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Received: January 16, 2002; accepted: May 16, 2002

Schlussfolgerungen: Ionisierende Strahlung reduziert die Expression von hTERT und Telomeraseaktivität. Diese Reduktion ist nicht allein auf die Induktion von Apoptose oder Nekrose zurückzuführen. Die Reduktion der Telomeraseaktivität kann zu einem verzögerten Zelluntergang nach Bestrahlung beitragen. Die kombinierte Verwendung von Bestrahlung und spezifischen Telomeraseinhibitoren ist ein potentiell synergistischer Therapieansatz.

Schlüsselwörter: Ewing-Tumor · Xenograft · Telomerase · Apoptose

Introduction

Telomerase, a ribonucleoprotein enzyme complex, forms specially structured ends of chromosomal DNA (TTAGGG)n called telomeres. Telomeres are responsible for the stabilization of the chromosome. In each mitosis, shorting of telomeres occurs which is known as the end replication problem. When telomeres are shortened to a critical length, the cell is unable to undergo further mitoses and enters senescence. The mitotic capacity or the age of a cell can be estimated by the residual telomere length. Telomerase compensates for the physiological loss of telomeres and allows infinite replication of the cell. Furthermore telomerase also plays a role in chromosomal repair. It is active in fetal tissue; however, from the postnatal period on, it is only present in germinal cells and in stem cells of highly reproductive organs (intestine, bone marrow, etc.). Moreover, telomerase is active in most tumor cells [4, 14, 23]. Its activity has been predictive for the outcome for patients with neuroblastoma and helps to distinguish between malignant and benign cells in breast lesions [24, 25].

Suppression of telomerase activity is a promising anticancer methodology. Tumor cells with inhibited telomerase activity lose their infinite replication capacity. Repression of telomerase activity has been described for some cytostatic drugs and for substances inducing cell differentiation such as retinoids [3, 6, 12, 16, 33]. Furthermore specific telomerase inhibitors are under investigation including antisense oligonucleotides, hammerhead ribozymes and small molecules [9, 18, 34]. There are few reports in the literature that comment about changes of telomerase activity after the application of ionizing radiation [11, 15, 26, 30]. Like chemotherapeutic agents, radiation may have the potential to reduce telomerase activity and, therefore, reduce the proliferative capacity of tumor cells. Furthermore changes of telomerase activity could be useful as a parameter to predict the effect of radiation [22]. Residual telomerase activity after irradiation may indicate that radioresistent cells were able to survive radiation-induced cell damage. In this analysis we have investigated the effect of radiation on telomerase activity in an Ewing xenograft tumor.

Material and Methods

Source of Tumor

The Ewing tumor cell line STA-ET-1 was classified as a malignant peripheral neuroectodermal tumor and kindly provided by P. Ambros, CCRI, Vienna. It shows a chromosomal t(11/22) translocation EWS-FLI1 (type I) and a TP53 exon 6 mutation [1, 5, 7].

Xenograft Mouse Model for Solid Tumors

All tumors grew subcutaneously as solid tumors in athymic mice (nu/nu). Initially, 5×10^6 cells (in 500 µl suspension) were injected subcutaneously in the flank. The tumor was then transplanted subcutaneously with an approximate volume of 8 mm^3 into the thigh of an immundeficient mouse. Take rate, tumor doubling time, and histologic analyses were performed for each transplanted tumor.

Radiation

Electron irradiation of the tumor was performed with 5 Gy and 30 Gy on a linear accelerator in our department. Initial dosimetric evaluations were performed to guarantee a homogenous dose distribution. A special immobilization device was used that allows focused irradiation of the tumor-bearing thigh without irradiation of the entire mouse. The animals need not be anesthetized for the procedure. Initially, the dose-response relationship for the tumor was established [13]. Experiments about telomerase activity were performed with 5 Gy and 30 Gy. After irradiation with 5 Gy, tumors were examined 6, 12, 24, 48, 72, and 96 hours post irradiation. After irradiation with 30 Gy, tumors were examined 6,12, 24, 48, 72 and 96 hours post irradiation and additionally after 6, 9, 12 and 15 days. For each dose and time group, three tumors were evaluated. Telomerase activity was measured in two independent experiments.

Protein Preparation and Telomerase Assay for the Measurement of Telomerase Activity

Tumor material was extracted and stored at -80 °C until use. Each sample was imbedded in paraffin to allow immunohistologic examination. A tumor content exceeding 70% was mandatory for all of the samples included in this study. Extractions of cellular proteins of Ewing tumor specimens were prepared as previously described by using CHAPS buffer. For in-vitro detection of telomerase activity, a modified version of the TRAP assay was used [23]. The accuracy of this quantitative analysis was further determined by several dilution series.

Assessment of Tissue Sample Preservation by RT-PCR of RNA Content

To minimize the probability of false-negative results with a lack of or low telomerase activity resulting from tissue degradation and necrosis, RNA derived from frozen sections and corresponding to the tumor material subjected to the TRAP procedure was amplified by RT-PCR for GAPDH as an indirect marker of tissue integrity. Briefly, a 297-base pair fragment of the human GAPDH gene was amplified with primers 5′-CACCCATGGCAAATTCCATGGC-3′ and 5′-GCATTGCTGATGATCTTGAGGCT-3′, corresponding to GenBank positions 213–234 and 509–487, respectively (Gen Bank accession no. M33197). All specimens were positive for GAPDH mRNA.

Quantitative Real-Time RT-PCR for Expression of the Telomerase Subunits hTERT, hTR, and TP1

Total RNA was isolated from fresh-frozen tissue using the TRISOLV system (Biozol, Eching, Germany) in accordance with the manufacturer's protocol. RNA was treated with DNase (Eurogentec, Seraing, Belgium) and purified with the RNeasy Mini Prep Kit (Qiagen, Hilden, Germany). cDNA was synthesized from approximately 2 µg of RNA using the FirstStrand Synthesis Kit (Amersham Pharmacia, Freiburg, Germany) with dT18 primers. Relative concentrations of cDNA samples were evaluated by quantitative RT-PCR of GAPDH performed on the LightCycler (Roche Diagnostics) followed by analysis of the gene expression of each telomerase subunit by the same procedure as previously described [23, 27]. Quantitative analysis was performed using the LightCycler Software (Roche Diagnostics) using a real-time fluorogenic detection system for a kinetic rather than endpoint approach as on conventional agarose or polyacrylamide gels. The generation of quantitative data was based on different PCR kinetics of samples with different levels of target gene expression. We used a relative quantification in which the expression levels of the tumor samples were compared to the data from telomerase-positive Ewing tumor cell line VH64 in a geometric dilution series (1 : 1, 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32, 1 : 64). The graph of the linear regression and calculation of the regression coefficient r served to confirm the accuracy and reproducibility of this approach. hTERT (human telomerase reverse transcriptase) expression was measured with the Roche Diagnostics kit based on PBGD levels, whereas hTR (human telomerase RNA) and TP1 (telomerase-associated protein) was measured based on GAPDH levels. For analysis, the quantitative amounts of hTERT, hTR, and TP1 gene expressions were calculated by dividing the measured amount by the expression of PBGD or of GAPDH.

The identity and specificity of the PCR product was confirmed by melting curve analysis, which is part of the LightCycler analysis program. The specific melting point of the PCR product for TP1 and hTR was correlated with its molecular weight as determined by agarose gel electrophoresis and fragment length analysis on an automated laser-fluorescence sequencer (ALFexpress, Pharmacia).

Growth Fraction

Ki-67 immunostaining and proliferation index: Routinely formalin-fixed and paraffin-embedded tissue was cut (3 µm) and proliferative activity was determined immunohistochemically using mouse monoclonal MIB 1-antibody (Dianova, Hamburg, Germany).

Cells of the lesions with distinct red nuclear staining were regarded as MIB 1 positive irrespective of the intensity of the reactions. Cell counts were made at 400-fold magnification using a 10 × 10 eyepiece grid on a Zeiss Axioscope microscope (Zeiss, Goettingen, Germany) in at least five neighboring viewing fields (yielding 500–1,000 cells). The MIB 1 labeling index (LI) was determined as the percentage of positive cells in the total number of cells counted in each case.

Apoptosis

The kryo-fixed tissue was analyzed using TUNEL-Assay according to the manufacturer (Apoptag-Peroxidase In Situ Apoptosis Detection Kit, Intergen, Oxford, UK).

The percentage of apoptotic cells (apoptotic index = number of TUNEL-positive cells out of all positive and negative cells) was determined by counting about 300–400 tumor cells in five randomly chosen areas of each sample on a light microscope at 40× magnification.

Necrosis

Areas of necrosis were defined by estimating the necrotic areas in HE stained histologic slides in relation to the complete sample using a raster ocular (in percent).

Statistics

The statistical significance of telomerase activity, growth fraction, expression of hTERT, TP1 and hTR at different times after radiation were calculated with one-way analysis of variance. For each time point, three different tumors were measured, additionally a repeat experiment was performed for telomerase activity. All these values were used to calculated statistical significance.

Results

Initial experiments about radiation-induced growth retardation showed a TCD 50 (dose which controls 50% of the tumors) of 47 Gy for STA-ET-1 xenograft tumors. Radiation with 10 Gy or below showed no relevant growth retardation (Figure 1). In tumors irradiated with 30 Gy, growth retardation was observed but no complete remission was induced in any of the tumors (Figure 2).

After irradiation with 5 Gy, the irradiated tumors showed a minor increase of telomerase activity within the first 12 hours followed by a decrease of telomerase activity. The max-

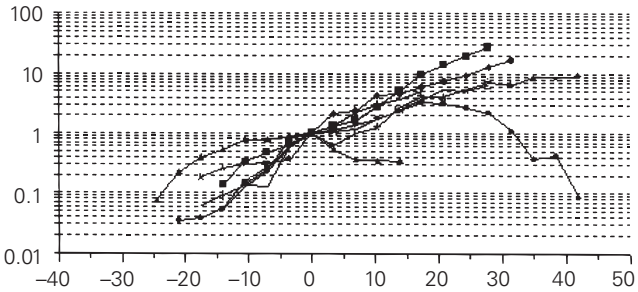

Figure 1. Relative volume of the xenograft tumor STA-ET-1 before and after irradiation (day 0) with 10 Gy in seven animals (time axis in days).

Abbildung 1. Relatives Volumen des Xenografttumors STA-ET-1 nach Bestrahlung mit 10 Gy bei sieben Tieren (Zeitachse in Tagen).

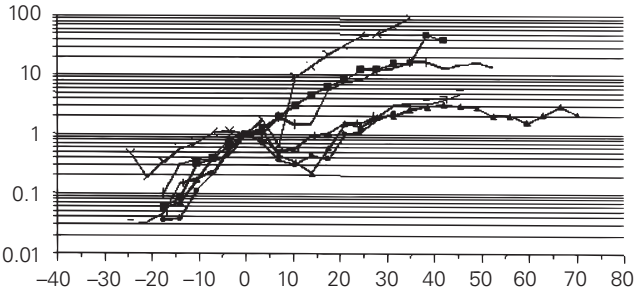

Figure 2. Relative volume of the xenograft tumor STA-ET-1 before and after irradiation (day 0) with 30 Gy in six animals (time axis in days).

Abbildung 2. Relatives Volumen des Xenografttumors STA-ET-1 nach Bestrahlung mit 30 Gy bei sechs Tieren (Zeitachse in Tagen).

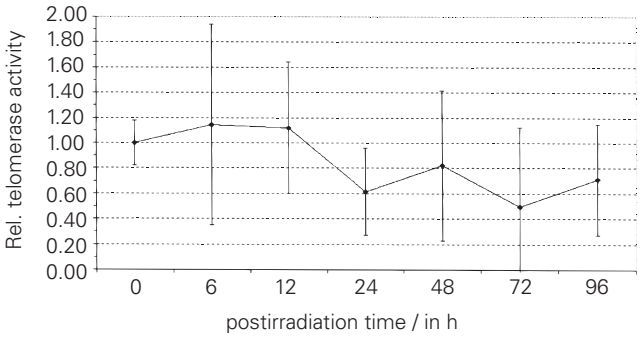

Figure 3a. Telomerase activity after irradiation with 5 Gy. The difference is not statistically significant. At each time point, three tumors were examined. Each telomerase activity value was measured in two different experiments.

Abbildung 3a. Telomeraseaktivität nach Bestrahlung mit 5 Gy. Der Unterschied ist statistisch nicht signifikant. Pro Zeitpunkt wurden drei Tumoren untersucht. Die Telomeraseaktivität wurde in je zwei unabhängigen Versuchen gemessen.

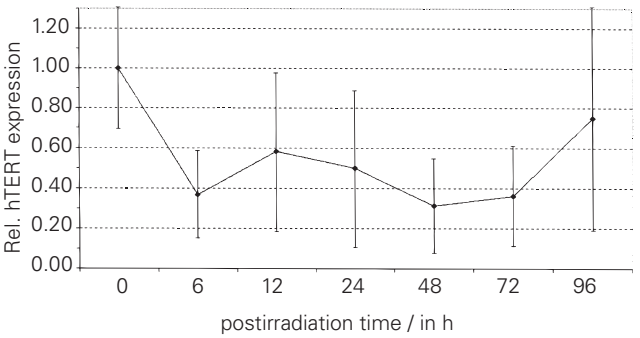

Figure 3b. Expression of hTERT after irradiation with 5 Gy. The changes of the expression of hTERT after irradiation compared with unirradiated controls are statistically significant (p = 0.013). At each time point, three tumors were examined.

Abbildung 3b. Expression von hTERT nach Bestrahlung mit 5 Gy. Die Änderung der Expression von hTERT im Vergleich zu unbestrahlten Kontrollen ist statistisch signifikant (p = 0,013). Pro Zeitpunkt wurden drei Tumoren untersucht.

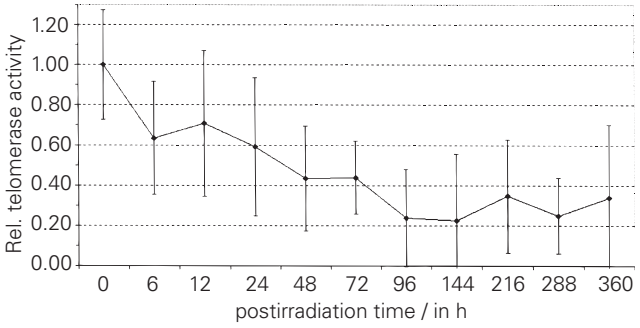

Figure 4a. Telomerase activity after irradiation with 30 Gy. The changes of telomerase activity are statistically significant (p = 0.001). At each time point, three tumors were examined. Each telomerase activity value was measured in two different experiments.

Abbildung 4a. Telomeraseaktivität nach Bestrahlung mit 30 Gy. Die Änderung der Telomeraseaktivität im Vergleich zu unbestrahlten Kontrollen ist statistisch signifikant (p = 0,001). Pro Zeitpunkt wurden drei Tumoren untersucht. Die Telomeraseaktivität wurde in je zwei unabhängigen Versuchen gemessen.

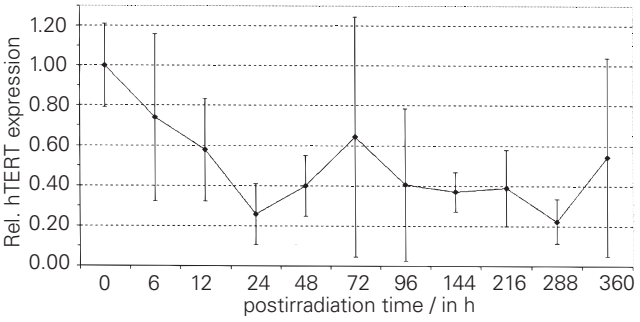

Figure 4b. Expression of hTERT after irradiation with 30 Gy. The changes of hTERT are statistically significant (p = 0.022). At each time point, three tumors were examined.

Abbildung 4b. Expression von hTERT nach Bestrahlung mit 30 Gy. Die Änderung der Expression von hTERT im Vergleich zu unbestrahlten Kontrollen ist statistisch signifikant (p = 0,022). Pro Zeitpunkt wurden drei Tumoren untersucht.

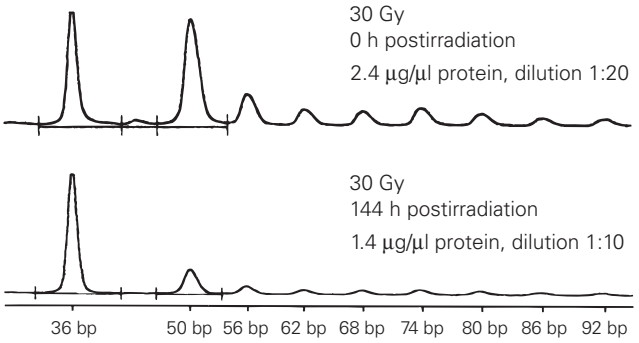

Figure 5. Results of the TRAPeze assay for an unirradiated control tumor and after 30 Gy at day 6. The peaks at base pair (bp) 50+ represent the amplification of telomerase products. The amplification is competitive to the 36 bp product. A high 50 bp peak compared to the 36 bp peaks correlates with high telomerase acitivity.

Abbildung 5. Ergebnis des TRAPeze-Assays für einen unbestrahlten Kontrolltumor sowie nach 30 Gy und 6 Tagen. Die Kurven an den Basenpaaren (bp) 50+ zeigen die Amplifikation der Telomeraseprodukte. Die Amplifikation läuft kompetitiv zum 36-bp-Produkt ab. Eine hohe 50-bp-Kurve im Vergleich zur 36-bp-Kurve korreliert mit einer hohen Telomeraseaktivität.

imal decrease of telomerase activity was observed after 72 hours with a reduction of 50%. The difference in telomerase activity was not statistically significant in the one-way analysis of variance compared to unirradiated controls within the observed 96 hours (Figure 3a). hTERT correlated with telomerase activity and showed a reduction after 5 Gy (Figure 3b). The reduction of hTERT was statistically significant ($p = 0.013$). The expression of hTR and TP1 increased after irradiation compared to unirradiated controls (data not shown).

After irradiation with 30 Gy, a growth retardation occurred without induction of a complete remission (see Figure 2). Telomerase activity was reduced by a factor of 4 with the maximal reduction occurring 6 days after irradiation (Figure 4a). After more than 6 days post radiation there was a slight restitution of telomerase activity. The expression of hTERT correlated with telomerase activity (Figure 4b). The reductions in telomerase activity and hTERT were statistically significant ($p = 0.001$ and $p = 0.022$, respectively). The peaks of the TRAPeze assay for a control tumor and after irradiation with 30 Gy at day 6 are shown in Figure 5. As with 5 Gy there was an increase in the expression of hTR and TP1 after irradiation but there was no correlation with changes in telomerase activity (data not shown). The changes in growth fraction after irradiation with 30 Gy are summarized in Figure 6. After irradiation, there was a reduction of growth fraction by 50% and a complete restitution after 15 days. The changes in growth fraction were statistically significant ($p < 0.001$). The MIB 1-positive areas were evenly distributed on the histologic slides, there was no focal enhancement of MIB 1-positive cells (Figure 7a). Areas of necrosis were estimated with a raster ocular (Figure 7c). Maximal necrosis after the application of 30 Gy was observed after 6 hours to be 19%. The maximum fraction of apoptotic cells after irradiation with 30 Gy was 6.9%. Figure 7b shows apoptosis in an immunostained histologic slide 6 days after irradiation. The percentage of necrotic and apoptotic cells and growth fraction are shown in Figure 6.

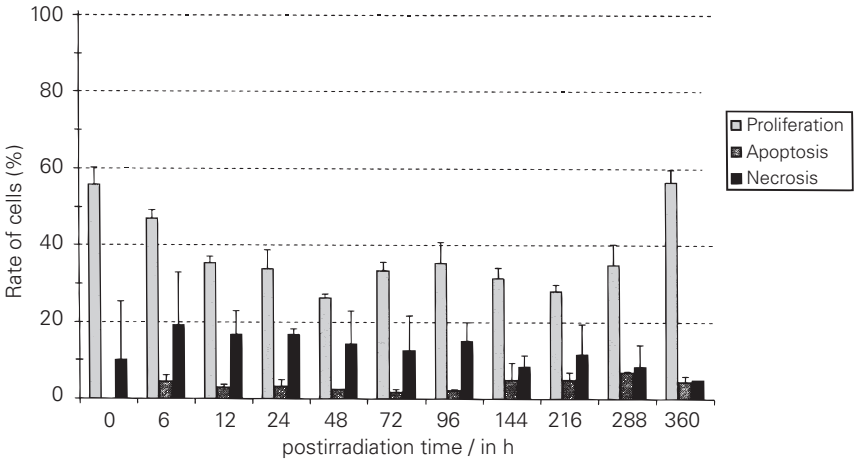

Figure 6. Growth fraction (MIB 1), rate of apoptosis and of necrosis after irradiation with 30 Gy. At each time point, three tumors were examined.

Abbildung 6. Wachstumsfraktion (MIB 1), Apoptose- und Nekroserate nach Bestrahlung mit 30 Gy. Pro Zeitpunkt wurden drei Tumoren untersucht.

Discussion

The impact of ionizing radiation on telomerase activity in tumor cells is not well characterized. The first effects of ionizing radiation on telomerase activity were published by Leteurtre et al [15]. Hematopoetic cells received 0,5–4 Gy in vitro. Within the observed 24 hours after the application of radiotherapy, telomerase activity increased. Similar results with an initial increase of telomerase activity after irradiation were reported for human colon carcinoma, lymphoma and myeloma cell lines [11, 21, 30]. Sawant et al [26] examined the changes of telomerase activity after irradiation of telomerase-positive cells of human cervical carcinoma and colorectal carcinoma. The latter cell line was additionally established as a human xenograft tumor on NMRI mice. In vitro, a dose- and time-dependent reduction of telomerase activity was observed in plateau phase cells. Higher doses and a longer period after radiation were associated with a more marked reduction of telomerase activity. In exponentially

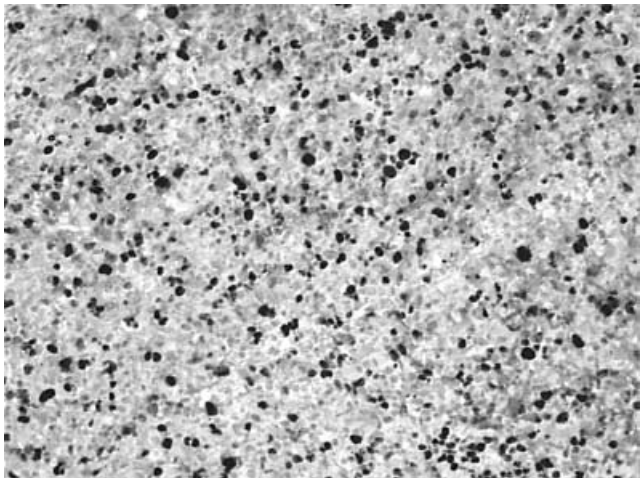

Figure 7a. Growth fraction (MIB 1 immunohistochemistry) 6 days after irradiation with 30 Gy: growth fraction of about 30% of the tumor cells.

Abbildung 7a. Wachstumsfraktion (MIB 1) 6 Tage nach Bestrahlung mit 30 Gy: Wachstumsfraktion von etwa 30%.

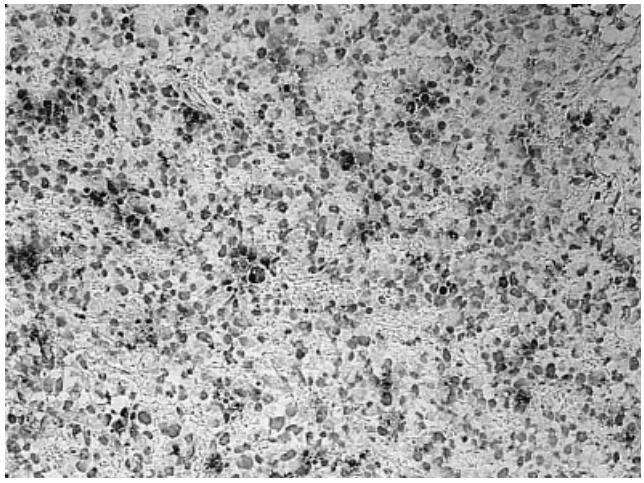

Figure 7b. Apoptosis 6 days after irradiation with 30 Gy: approximately 5% of the tumor cells with nucleus staining (TUNEL assay).

Abbildung 7b. Immunhistologischer Nachweis von Apoptose 6 Tage nach Bestrahlung mit 30 Gy bei etwa 5% der Tumorzellen.

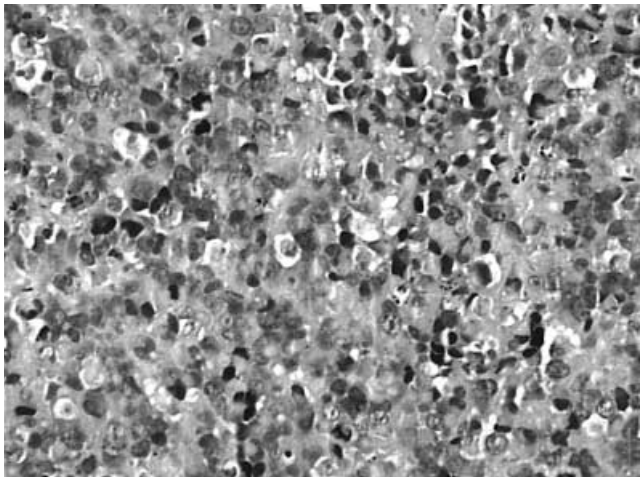

Figure 7c. Necrosis 6 days after irradiation with 30 Gy: approximately 10% of the whole area with necrosis.

Abbildung 7c. Nekroseareale 6 Tage nach Bestrahlung mit 30 Gy von etwa 10%.

growing HeLa cells, an initial increase in telomerase activity was seen with a maximum at 72 hours after radiation of 10 Gy followed by a decrease in telomerase activity below the level of unirradiated controls. In tumor samples from xenograft tumors irradiated with 25 Gy, a maximum reduction of telomerase activity was observed after 10 days with a subsequent restitution of telomerase activity in tumors that did not show a complete remission. Lin et al [16] observed an inhibition of telomerase activity after irradiation in two of three lymphoma cell lines.

In the present study, we observed a minor initial increase of telomerase activity within the first 12 hours post irradiation with 5 Gy. Then a reduction of telomerase activity of 50% was observed in the Ewing xenograft tumor (see Figure 3a) although the differences in telomerase activity were not statistically significant in the one-way analysis of variance. The reduction of telomerase activity was paralleled by a reduction of the expression of hTERT (see Figure 3b), the decrease of hTERT expression was statistically significant [2]. There was an increase in the expression of hTR and TP1 after the application of 5 Gy radiation. The changes in the expression of these genes did not correlate with telomerase activity. An initial increase of telomerase activity after a moderate dose of radiation would be in line with the observations of other investigators [11, 15, 26, 30].

The application of sublethal doses of 30 Gy resulted in a decrease of telomerase activity by a factor of 4 with a maximum reduction after 6 days (see Figure 4a). The decrease of telomerase activity correlated with the expression of hTERT (see Figure 4b) and growth fraction (see Figure 6), but it did not correlate with hTR or TP1 expression. The changes in telomerase activity, hTERT expression and growth fraction are statistically significant. Between day 6 and day 15 post irradiation, telomerase activity remained stable. The time between irradiation and minimum telomerase activity was comparable to the in-vivo results by Sawant et al [26] who observed minimal telomerase activity 10 days after irradiation with 25 Gy.

These results and those of other investigators show that radiation results in a dose-dependent inhibition of telomerase activity in tumor cells if observed for a longer period of time. This is paralleled by a reduction of the expression of hTERT and a reduction of growth fraction. It is unlikely that radiation

can specifically reduce telomerase activity. It is probable that the reduction of telomerase activity is due to complex changes within the damaged cell after radiation that involve the expression of the catalytic subunit of telomerase hTERT, and consequently telomerase activity. The changes of telomerase activity cannot be explained by induction of necrosis or apoptosis alone since only a small portion of cells were undergoing apoptosis or were necrotic after irradiation with 30 Gy (Tables 1 and 5 in [31]). Furthermore the expression of hTR and TP1 increased during the observation period. This demonstrates that cells were still viable and metabolically active.

Radiation is known to have a rapid effect on tumor cells by inducing irreparable DNA damage and causing cell death. Apart from its direct effect, ionizing irradiation can induce delayed reproductive death due to genomic instability [10, 17, 19, 20, 28, 29]. There is growing evidence that cells which survive ionizing irradiation may exhibit induced genomic instability, delayed gene mutations and delayed reproductive death in progeny that appeared to be normal healthy cells until the cell division which resulted in a lethal event [20]. Inhibition of telomerase activity and subsequent shortening of telomeres in telomerase activity-negative cells lead to chromosomal instability [27] and senescence. It would therefore be possible that inactivation of telomerase activity by ionizing irradiation is one mechanism that causes the known post-irradiation genetic instability and delayed cell death. The combination of specific telomerase inhibitors such as antisense oligonucleotides, hammerhead ribozymes and small molecules [9, 18, 34] with ionizing irradiation may have two potential synergistic effects. A more potent reduction of telomerase activity could be induced by using specific telomerase inhibitors and radiation. Furthermore radiation can induce double strand breaks in the repetitive telomere sequence. Together with a reduction of telomerase activity by specific inhibitors, critical shortening of telomeres and subsequent senescence would occur more rapidly. This phenomenon has been reported by Goytisolo et al [8] who observed that short telomeres resulted in organismal hypersensitivity to ionizing irradiation in mammals. Mice with telomeres 40% shorter than wild-type controls showed higher chromosomal damage and greater apoptosis after irradiation than control animals. This hypersensitivity syndrome to irradiation was also observed in late generation telomerase negative mice by a different group [32]. The radiosensitivity of telomere dysfunctional cells correlated with delayed DNA break repair kinetics, persistent chromosomal breaks and cytogenetic profiles characterized by complex chromosomal aberrations and massive fragmentation. The promising combined effect of specific telomerase inhibition and radiation in vitro and in vivo is currently under investigation in our group.

Acknowledgment

This study was supported by a grant from the Federal Ministry of Education and Research (Fö.01KS9604/04) and the Interdisciplinary Center of Clinical Research Muenster (IZKF Project No. 3H2).

References

1. Becker A, Stadler P, Krause U, et al. Keine Assoziation zwischen p53-Überexpression und polarisch gemessener Tumoroxygenierung bei Patienten mit Kopf-Hals-Karzinomen. Strahlenther Onkol 2000;176:475–80.
2. Beck-Bornholt HP, Dubben HH. Multiple Signifikanztests und ihre Bedeutung bei der Beurteilung von Resultaten. Strahlenther Onkol 2000;176: 344–9.
3. Burger AM, Double JA, Newell DR. Inhibition of telomerase activity by cisplatin in human testicular cancer cells. Eur J Cancer 1997;33:638–44.
4. Counter CM, Avilion A, LeFeuvre CE, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 1992;11:1921–9.
5. Dahm-Daphi J. p53: Biology and role for cellular radiosensitivity. Strahlenther Onkol 2000;176:278–85.
6. Faraoni I, Turriziani M, Masci G, et al. Decline in telomerase activity as a measure of cell killing by antineoplastic agents in vitro. Clin Cancer Res 1997;3: 579–85.
7. Finke C, Rübe C, van Valen F, et al. Time and dose dependent upregulation of TNF-alpha after irradiation of Ewing's tumor cells in vitro and in vivo. Radiother Oncol 1996;40:176.
8. Goytisolo FA, Samper E, Martin-Caballero J, et al. Short telomeres result in hypersensitivity to ionizing irradiation in mammals. J Exp Med 2000;192: 1625–36.
9. Hahn W, Stewart S, Brooks M, et al. Inhibition of telomerase limits the growth of human cancer cells. Nature Med 1999;5:1164–70.
10. Hendry JH. Genomic instability: potential contributions to tumour and normal tissue response, and second tumours, after radiotherapy. Radiother Oncol 2001;59:117–26.
11. Hyeon J, Hande M, Lansdorp P, Natarajan A. Induction of telomerase activity and chromosome aberrations in human tumor cell lines following irradiation. Mutat Res 1998;401:121–31.
12. Ishibashi T, Lippard SJ. Telomere loss in cells treated with cisplatin. Proc Natl Acad Sci USA 1998;95:4219–23.
13. Karger C, Hartmann G. Determination of tolerance dose uncertainties and optimal design of dose response experiments with small animal numbers. Strahlenther Onkol 2001;177:37–42.
14. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994;266: 2011–5.
15. Leteurtre F, Li X, Gluckman E, Carosella ED. Telomerase activity during the cell cycle and in gamma-irradiated hematopoetic cells. Leukemia 1997;11: 1681–9.
16. Lin Z, Lim S, Viani M, Sapp M, Lim MS. Down regulation of telomerase activity in malignant lymphomas by radiation and chemotherapeutic agents. Am J Pathol 2001;159:711–9.
17. Little J, Gorgojo L, Vetrovs H. Delayed appearance of lethal and specific gene mutations in irradiated mammalian cells. Int J Radiat Oncol Biol Phys 1990;19:1425–9.
18. Mata J, Joshi S, Palen B, et al. A hexameric phosporothioate oligonucleotide telomerase inhibitor arrests growth of Burkitt's lymphoma cells in vitro and in vivo. Toxicol Appl Pharmacol 1997;144:189–97.
19. Mothersill C, Kadhim MA, O'Reilly S, et al. Dose- and time-response relationship for lethal mutations and chromosomal instability induced by ionizing radiation in an immortalized human keratinocyte cell line. Int J Radiat Biol 2000;76:799–806.
20. Mothersill C, Kadhim MA, O'Reilly S, et al. Expression of delayed toxicity and lethal mutations in the progeny of human cells surviving exposure to radiation and other environmental mutagens. Int J Radiat Biol 1998;74: 673–80.
21. Neuhof D, Ruess A, Wenz F, Weber KJ. Induction of telomerase activity by irradiation in human lymphoblasts. Radiat Res 2001;155:693–7.
22. Ogawa Y, Nishioka A, Hamada N, et al. Changes of telomerase activity of advanced cancers of the oral cavity and oropharynx during radiation therapy: Correlation with clinical outcome. Int J Mol Med 1998;2: 301–7.
23. Poremba C, Scheel C, Hero B, et al. Telomerase activity and telomerase subunits gene expression patterns in neuroblastoma – A molecular and immunohistochemical study establishing prognostic tools for fresh-frozen and paraffin-embedded tissues. J Clin Oncol 2000;18:2582–92.

24. Poremba C, Shroyer K, Frost M, et al. Telomerase is a highly sensitive and specific molecular marker in fine needle aspirates of breast lesions. J Clin Oncol 1999;17:2020–6.
25. Poremba C, Willenbring H, Hero B, et al. Telomerase activity distinguishes between neuroblastomas with good and poor prognosis. Ann Oncol 1999; 10:715–21.
26. Sawant S, Gregoire V, Dhar S, et al. Telomerase activity as a measure for monitoring radiocurability of tumor cells. FASEB 1999;13:1047–54.
27. Scheel C, Schaefer KL, Jauch A, et al. Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcoma. Oncogene 2001;20:3835–44.
28. Scott D. Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. Strahlenther Onkol 2000;176:229–34.
29. Seymour CB, Mothersill C, Alper Z. High yields of lethal mutations in somatic mammilian cells that survive ionizing irradiation. Int J Radiat Biol 1986;50:167–79.
30. Terashima M, Ogawa Y, Toda K, et al. Effects of radiation on telomerase activity in human lymphoma and myeloma cell lines. Int J Mol Med 1998; 2:567–71.
31. Verheye-Dua F, Böhm L. Influence of apoptosis on the enhancement of radiotoxicity by Ouabain. Strahlenther Oncol 2000;76:186–91.
32. Wong KK, Chang S, Weiler S, et al. Telomere dysfunction impairs DNA repair and enhances sensitivity to ionizing irradiation. Nat Genet 2000;26:85–8.
33. Xu D, Gruber A, Peterson C, Pisa P. Suppression of telomerase activity in HL60 cells after treatment with differentiating agents. Leukemia 1996;10: 1354–7.
34. Yokoyama Y, Takahashi Y, Shinohara A, et al. The 5′-end of hTERT mRNA is a good target for hammerhead ribozyme to suppress telomerase activity. Biochem Biophys Res Commun 2000;273:316–21.

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