J Cancer Res Clin Oncol (1998) 124: 165–171


ORIGINAL PAPER

**Regine Schneider-Stock · Cornelia Epplen
Kathrin Radig · Yoshinao Oda · Henning Dralle
Cuong Hoang-Vu · Jörg T. Epplen · Albert Roessner**

# On telomere shortening in soft-tissue tumors

Received: 4 August 1997 / Accepted: 6 December 1997

**Abstract** Purpose: Specific simple DNA repeats occur at the telomeric ends of mammalian chromosomes. Loss of (G + C)-rich repeats can result in genetic instability, associated with tumorigenesis. So far, data on telomere shortening have not been available for different types of soft-tissue tumors. Methods: Using tumor material and the blood of the corresponding patient, high-molecular-mass DNA was prepared by digestion with proteinase K and extraction with phenol/chloroform. A 10-μg sample of DNA was digested with the restriction enzyme *Hin*fI. DNA fragments were separated in a 0.7% agarose gel, and in-gel hybridization was performed with the telomere-specific repeat probe $(TTAGGG)_3$. Results: Shortening of the telomere repeat was observed in 14/30 soft-tissue tumors; 5 tumors showed elongated telomere repeats, whereas the telomeres appeared unchanged in 11 tumors. Decreased telomere repeat length correlated with advanced age, DNA ploidy, and a higher proliferation index. There was no association between telomere repeat length and tumor grade. Interestingly, in contrast to other entities, all malignant schwannomas and leiomyosarcomas showed significantly reduced telomere lengths. An explanation for the telomere heterogeneity in liposarcomas may include differential telomerase reactivation in well and poorly differentiated tumors. Conclusions: Telomere shortening is frequent but not a uniform phenomenon in different types of soft-tissue tumor. Studies on telomerase activity should be performed in the same cohort of sarcomas.

**Key words** Telomeres · Soft-tissue tumors · Telomerase

**Abbreviations** *TRF* terminal restriction fragments · *MFH* malignant fibrous histiocytoma

R. Schneider-Stock (✉) · K. Radig · A. Roessner
Otto-von-Guericke University, Department of Pathology,
Leipziger Str. 44, D-39120 Magdeburg, Germany
e-mail: Regine.Schneider-Stock@medizin.uni-magdeburg.de
Tel.: +49 391 67 15060
Fax: +49 391 67 15818

C. Epplen · J. T. Epplen
Department of Molecular Human Genetics,
Ruhr-University, Bochum, Germany

Y. Oda
Department of Orthopedic Surgery,
Kyushu University, Fukuoka, Japan

H. Dralle · C. Hoang-Vu
Department of Surgery, Martin-Luther-University,
Halle, Germany

## Introduction

Human telomeres (terminal chromosome regions) are made up of several thousand copies of a repeating nucleotide sequence $(TTAGGG)_n$. These highly conserved sequences attach directly to the nuclear membrane, suggesting that telomeres may protect chromosome ends against illegitimate recombination and degradation (Vaziri and Benchimol 1996). Progressive shortening of human telomeres was noted with increasing age in blood (Allsopp et al. 1992), colonic mucosa (Hastie et al. 1990), and fibroblasts (Harley et al. 1990), perhaps reflecting reduced replicative capacity (Blackburn 1991).

Although the biological significance of this telomeric length reduction is unclear, it is possibly related to an increased frequency of chromosome abnormalities (Counter et al. 1992). Thus, telomere shortening contributes to genetic instability, an important mechanism in tumorigenesis. Recently, reductions in telomere repeat length have been detected in various solid tumors, e.g. colorectal cancer (Hiyama et al. 1995), ovarian cancer (Counter et al. 1994), giant-cell tumors of bone (Schwartz et al. 1995), and neuroblastomas (Hiyama et al. 1992). However, tumor cells seems to escape from progressive telomere shortening by expressing telomerase activity, an enzyme that catalyzes de novo production of telomeres by adding $(TTAGGG)_n$ repeats (Morin, 1990; Hiyama et al. 1996; Counter et al. 1995). We aimed to document further genetic abnormalities in soft-tissue tumors by determining telomere

lengths in a group of 30 tumors. Possible correlations with DNA ploidy and proliferative activity were also investigated.

## Materials and methods

We investigated 30 malignant soft-tissue tumors from 28 patients (see Table 1: 13 liposarcomas, 5 malignant fibrous histiocytomas, 7 malignant schwannomas, 3 leiomyosarcomas, and 2 synovial sarcomas). In one case, additional tissue from a metastasis (tumor 12, 12A) and, in another case, the primary tumor and its first local recurrence were available (tumor 18, 18A). Immediately after surgery, the tumor material was snap-frozen and stored at −80°C until DNA preparation. After additional immunological investigations, soft-tissue tumors were classified on hematoxylin/eosin-stained sections (Enzinger and Weiss 1995) (Figs. 1A, 2A).

### DNA cytometry

DNA-cyto-metric measurements were performed by an interactive AHRENSACAS cytophotometry system on a MS-DOS-compatible computer for data of static cytometry. Measurements were performed on 7-μm-thick Feulgen-stained paraffin sections; 150 tumor cells were counted in each step. Myocytes or fibrocytes served as reference cells. DNA indices from 1.0+ to −0.2 were judged to indicate diploid cells, those lying outside this range aneuploid cells.

### Proliferation index (Ki-67)

After deparaffinization with xylene for 15 min and dehydration through a series of graded alcohols, the sections were pretreated in a microwave oven using 0.01 M citrate buffer (pH 6.0) for 3 × 10 min. Sections were incubated with primary antibody anti-Ki-67, MIB-1 (Oncogene Science, USA), diluted at a ratio of 1:30, for 60 min, and incubated in a humified chamber at 37°C. Staining was revealed using the alkaline phosphatase/anti-(alkaline phosphatase) technique.

MIB-1-positive cells were estimated by examining 40 high-power fields in representative areas with approximately 1000 cells. In cases of heterogeneous distribution of labeled cells, the region with the highest density of MIB-1-positive nuclei was considered the area of proliferative tumor activity (Figs. 1B, 2B). The ratio of positive-staining nuclei to the total number of tumor cells was expressed as the MIB-1 proliferation index (%).

### DNA preparation

High-molecular-mass DNA of the tumor and the patients' corresponding peripheral blood lymphocytes were prepared by digestion of proteinase K and phenol/chloroform extraction (Sambrook et al. 1989).

**Table 1** Clinic opathological data and findings of DNA fingerprint analysis in soft-tissue tumors. $</\ll$ telomere shortening, $>$ telomere elongation, $=$ no change in telomere length; *M* male, *F* female; −/+/++ no/one/two banding alteration(s) *A* following a tumor number indicates the same patient; *TRF* terminal restriction fragment length; *NA* not available

| Tumor | Age (years) | Sex | Grade | $(GGATTT)_n$ | TRF | $(GTG)_5$ | $(GACA)_4$ | Ki-67 (%) | DNA ploidy |
|---|---|---|---|---|---|---|---|---|---|
| Liposarcomas | | | | | | | | | |
| 1 | 68 | M | G1 | < | 6 | ++ | − | 17 | Diploid |
| 2 | 66 | M | G2 | > | 9 | − | − | 25 | Diploid |
| 3 | 65 | M | G1 | < | 5 | − | − | 14 | Diploid |
| 4 | 77 | F | G2 | > | 8.5 | − | + | 12 | Aneuploid |
| 5 | 63 | M | G2 | > | 12 | − | − | 7 | Aneuploid |
| 6 | 60 | M | G3 | < | 5.5 | − | − | 32 | Aneuploid |
| 7 | 63 | M | G2 | = | 8 | − | − | 14 | NA |
| 8 | 67 | M | G3 | > | 11 | − | − | NA | NA |
| 9 | 63 | M | G1 | < | 6 | − | − | NA | NA |
| 10 | 72 | M | G1 | = | 5 | − | − | NA | NA |
| 11 | 52 | F | G1 | = | 8.5 | − | − | NA | NA |
| 12 | 53 | M | G1 | = | 10 | − | − | NA | NA |
| 12A | 54 | M | G1 | > | 12 | − | − | NA | NA |
| Malignant schwannomas | | | | | | | | | |
| 13 | 44 | M | G2 | ≪ | 5 | + | − | 16 | Aneuploid |
| 14 | 49 | F | G2 | < | 4.5 | − | − | 31 | Diploid |
| 15 | 25 | F | G3 | = | 8 | − | − | 24 | Aneuploid |
| 16 | 44 | M | G2 | ≪ | 3.5 | + | − | 16 | Diploid |
| 17 | 52 | F | G2 | ≪ | 3 | ++ | − | NA | NA |
| 18 | 29 | M | G2 | ≪ | 3.5 | − | − | NA | NA |
| 18A | 30 | M | G2 | < | 3.5 | ++ | − | NA | NA |
| Malignant fibrous histiocytomas | | | | | | | | | |
| 19 | 56 | M | G3 | = | 10 | − | − | 14 | Polyploid |
| 20 | 26 | M | G3 | = | 9 | ++ | − | 28 | Aneuploid |
| 21 | 27 | F | G1 | = | 8 | − | − | 11 | Diploid |
| 22 | 26 | F | G3 | = | 8.5 | − | ++ | 8 | Aneuploid |
| 23 | 74 | M | G3 | = | 9 | + | − | 16 | Aneuploid |
| Leiomyosarcomas | | | | | | | | | |
| 24 | 79 | F | G2 | ≪ | 3.5 | + | − | 25 | Diploid |
| 25 | 73 | M | G3 | < | 5 | − | − | 20 | NA |
| 26 | 67 | M | G2 | < | 9 | + | − | 28 | NA |
| Synovial sarcomas | | | | | | | | | |
| 27 | 21 | F | G2 | < | 5 | − | − | 21 | Diploid |
| 28 | 22 | F | G3 | = | 9 | − | − | 27 | Diploid |

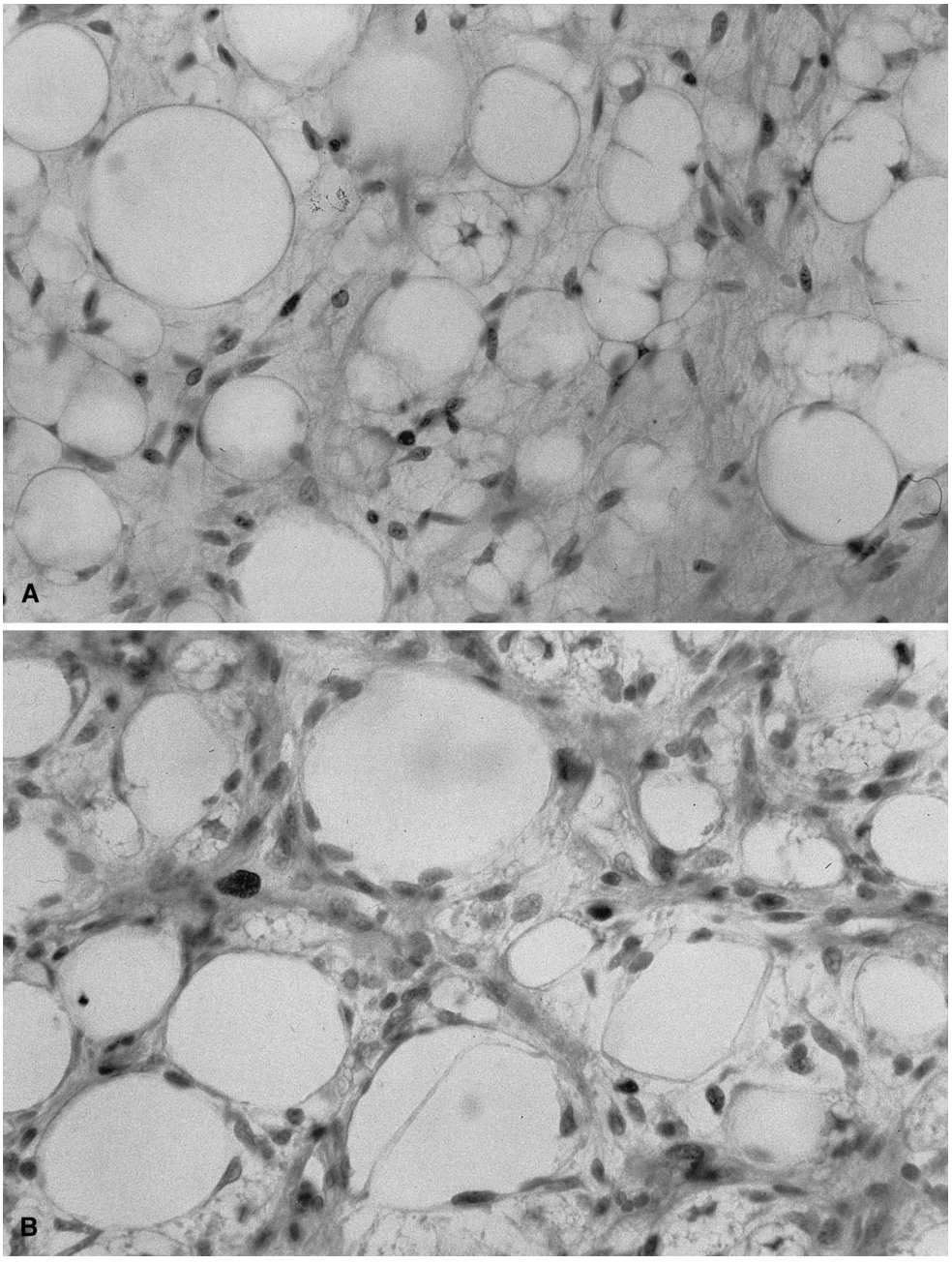


**Fig. 1** **A** Well-differentiated liposarcoma (tumor 1) composed of fat cells with moderate variations in size and shape and occasional multivacuolated lipoblasts (hematoxylin/eosin, H&E; ×470). **B** Amongst them broad fibrous septa containing spindle cells with positive reaction after incubation with monoclonal antibody MIB-1 (alkaline phosphatase/anti-(alkaline phosphatase, APAAP, method, ×470)

DNA restriction, electrophoresis and oligonucleotide hybridization

Samples containing 10 µg DNA were cut by using restriction endonuclease *Hin*fI (AGS, Heidelberg, Germany) according to the recommendations of the manufacturers. DNA fragments were run on a 0.7% agarose gel for 40 h at 1 V/cm. *Hin*dIII-digested lambda DNA (Promega, Madison, Wis., USA) was used as length standard. The agarose gel was dried for 1 h at 60°C under vacuum. In-gel hybridization was performed by using the telomere-specific probe $(TTAGGG)_3$ and the radioactive detection procedure (Epplen et al. 1991; Epplen 1992). In brief, gels were hybridized in 6 × standard saline/citrate (SSC; 1 × SSC is 0.15 M Nacl, 15 mM sodium citrate) at 43–45°C overnight and washed in 6 × SSC for 3 × 30 min at room temperature. Stringency washing was performed at the hybridization temperature for 1 × 1 min. The gels were also hybridized with the oligonucleotide probes $(GTG)_5$ and $(GACA)_4$ in order to identify possible changes as well as variations in DNA concentrations and quality of digestion. Terminal restriction fragments (TRF) are detected as smears of $(TTAGGG)_3$-hybridizing fragments of between $2 \times 10^3$ and $20 \times 10^3$ base pairs (kbp), see Fig. 3A. Telomeric signal values were measured by estimating the band size corresponding to the point with the highest absorbance within the peak. Whenever sufficient DNA was available, two independent hybridizations and evaluations were made.

Interpretation of results

In order to consider possible age-dependent telomere repeat shortening in blood lymphocytes, we judged variations in telomeric repeat lengths according to the following scheme: (=) no change in telomeric repeat lengths; (<) TRF lengths were shorter than 80%;(≪) TRF lengths were shorter than 50%; (>) telomeric repeat elongation of more than 120%

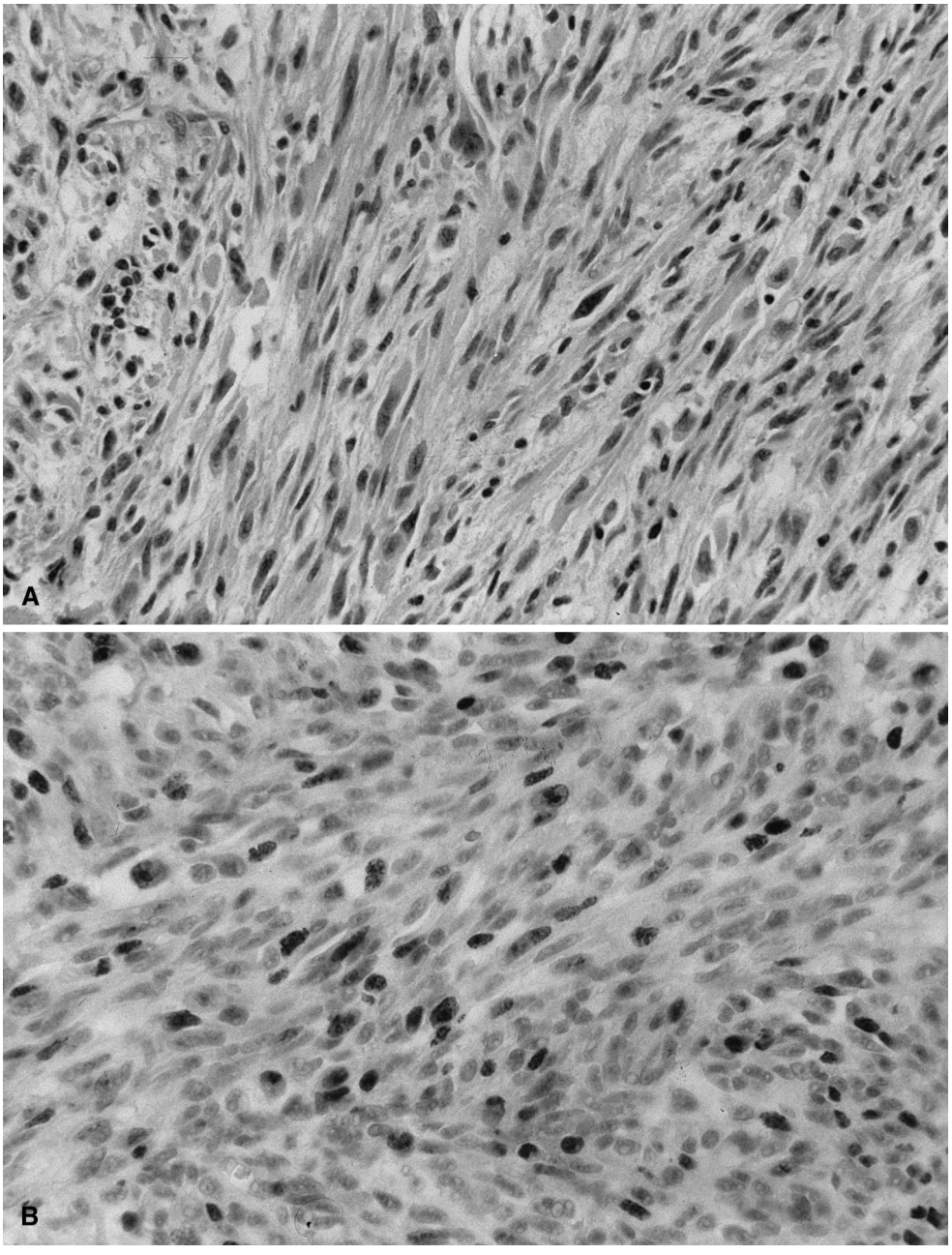


**Fig. 2 A** Moderately differentiated leiomyosarcoma (tumor 24) arranged in fascicles. Note the hypercellularity and focal nuclear pleomorphism of the spindle cells (H&E; ×360). **B** In this tumor immunohistochemical staining of MIB-1 showed a distinct nuclear reaction in most of the tumor cells (APAAP method; ×360)

Statistical analyses

For statistical analyses, the mean TRF was correlated with other clinicopathological data. Non-parametric tests were used throughout. The Kruskall-Wallis test was used for *n*-groups (tumor grade, age, tumor presentation, entities), whereas the Mann-Whitney *U*-test was used for paired groups (ploidy, DNA fingerprint differences). All *P* values resulted from two-sided tests.

## Results

### DNA fingerprint differences shown by hybridization with $(GTG)_5$ and $(GACA)_4$

On the basis of the hybridization patterns obtained with the repetitive oligonucleotide probe $(GTG)_5$, we excluded differences in quality in the DNA from tumor and non-neoplastic tissues. Differences in the DNA fingerprinting patterns between tumor tissue and the blood of the corresponding patients (Table 1) were noted in 11/30 soft-tissue tumors (36.7%). These differences comprised 1/8 GI tumors (12.9%), 7/13 GII tumors (53.8%), and 2/9 GIII tumors (22.2%). Most differences were detected with the oligonucleotide probe $(GTG)_5$ (9/11). Only two tumors showed altered banding patterns after hybridization with $(GACA)_4$.

Altered DNA fingerprint patterns (Table 1) were observed at a very high frequency in malignant schwannomas (4/7 tumors, 57%), in MFH (3/5 tumors, 60%) and in leiomyosarcomas (2/3 tumors, 60%). Changes were less common in liposarcomas (2/13 tu-

mors, 15%) and were not observed in the two synovial sarcomas. Interestingly, one primary malignant schwannoma (tumor 18) presented without changes, whereas the first local recurrence (tumor 18A) displayed two banding changes as compared to the patient's blood. There was no association between DNA fingerprint differences and age (53.4 years compared to 51.6 years), DNA ploidy status, or proliferative activity (18.4% compared to 20%), respectively. Figure 3B shows the hybridization pattern obtained with probe $(GTG)_5$. In one liposarcoma (tumor 1), an additional band in the 6-kb region was identified (Figs. 1A, B and Fig. 3B). Another supernumerary band was observed in a malignant schwannoma (tumor 17) and one band was missing simultaneously in the 3-kb range (+ + in Table 1). One MFH (tumor 23) displayed apparent amplification of two DNA fingerprint bands at 3.5 kb and 3 kb.

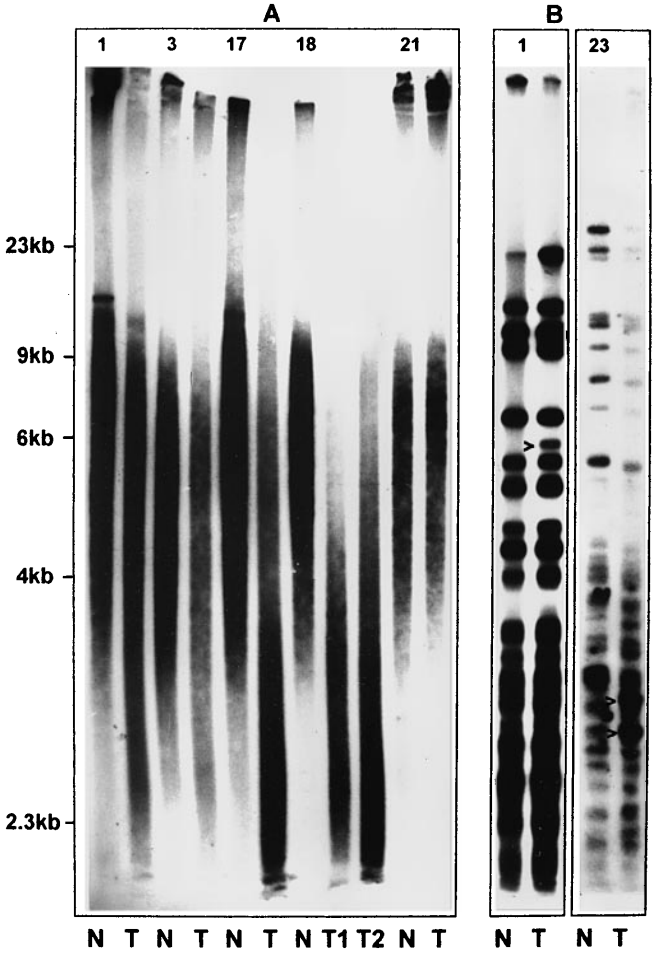


**Fig. 3** DNA fingerprint gels comparing DNA of non-tumor DNA (*N*) and tumor DNA (*T*) of a patient **A** Hybridization with the telomere-specific probe $(GGATTT)_3$. Tumors 1, 3, 17 and 18 showed telomere shortening; the local recurrence ($T_2$) of tumor 18 demonstrated a partly reconstituted telomeric length; tumor 21 showed no change in telomeric length in comparison to blood of this patient. **B** Hybridization with $(GTG)_5$. In tumor 1 an additional DNA fingerprint band was observed at 6 kb; tumor 23 showed a marked enhancement of signal intensity (probable amplification) for two bands at 3 kb and 3.5 kb

### Determination of TRF after hybridization with $(GGATTT)_3$

In 14/30 tumors, the telomeric repeat was less than that of the respective normal tissue (Table 1); among them were 4/8 GI tumors (50%), 9/13 GII tumors (69.2%), and 2/9 GIII tumors (22.2%). Telomere-repeat elongation was found in 5/30 (16.7%) tumors investigated. These malignomas comprised 1/8 GI tumors (12.5%), 3/13 GII tumors (23.1%) and 1/9 GIII tumors (11.1%) of the 30 tumours, 11 displayed no alteration in telomeric repeat length in tumor tissue, as compared with the patients' blood; among them were 4/8 GI tumors (50%), 1/13 GII tumors (7.6%), and 6/9 GIII tumors (66.7%).

Interestingly, in contrast to the primary tumor (tumor 12), the metastasis of this liposarcoma had restored the telomere repeat length to the basic level. The first local recurrence of a malignant schwannoma showed a partly reconstituted telomere length in comparison to the primary tumor (Fig. 3, tumor 18).

Telomeres were significantly shortened in malignant schwannomas. The mean TRF was 4.4 kb as compared with those of MFH (8.7 kb), liposarcomas (8.2 kb), leiomyosarcomas (5 kb), and synovial sarcomas (7 kb) ($P = 0.02$).

The proliferation indices (Ki-67) were determined for 20 tumors. Tumors with telomere shortening had higher proliferation indices (mean 21.8%) than tumors without any change (mean 18.3%) or with telomeric elongation (mean 14.7%). Diploid tumors exhibited significantly shorter TRF than aneuploid ones ($P = 0.04$).

## Discussion

As cells divide, the ends of the chromosomes, consisting of the simple TTAGGG repeats, erode progressively. According to one model, telomerase activity maintains telomeric length in the germline as well as in immortal cells and thus compensates for the "end-replication problem" (Kim et al. 1994). Many authors have described the loss of telomere DNA in human tumors (Hiyama et al. 1992; Nürnberg et al. 1993; DeLange et al. 1990). In general, our results support the findings described in the literature, according to which tumors show a reduction in the length of telomere repeats as compared to non-neoplastic reference tissues. Examining 30 soft-tissue tumors for changes in the length of their TRF, we found telomere-repeat reduction in 46.7% of the tumors. There was a significant difference between tumor entities, with malignant schwannomas showing the shortest telomeric repeats (4.4 kb) while MFH had increased telomeric lengths (8.7 kb). Homogeneous distribution patterns of TRF seem to be characteristic for both types: malignant schwannomas have exclusively shortened telomere repeats, whereas the telomere lengths of MHF were always unchanged. Liposarcomas, however, exhibited very heterogeneous distribution patterns of TRF, indicating that telomere

shortening is not a uniform feature in soft-tissue sarcomas.

Mehle et al. (1994) demonstrated the presence of different tumor cell clones of various telomere lengths in primary tumors of renal cell carcinomas. Royle (1996) hypothesized that telomerase-expressing cells are able to maintain telomeric lengths and genome composition. Therefore, these cells may have growth advantages over other non-expressing malignant cells and outgrow them. In our study, in the two cases where multiple samples were analyzed for telomeric length, there was an indication of a heterogeneous pattern (Table 1). In the primary tumor of a malignant schwannoma (tumor 18), the telomeric length was reduced drastically, whereas the telomeric repeat length was partly reconstituted because longer TRF were measured. For the primary tumor of a liposarcoma (tumor 12), the telomeric repeat length was unchanged, whereas telomeric elongation was noted in the recurrence.

A possible explanation for the TRF heterogeneity in liposarcomas may involve different periods of telomerase expression. Once tumor cells acquire telomerase activity, their TRF could be stabilized at any size. The balance between the loss of TRF at each cycle of DNA replication and the telomere elongation due to telomerase activity may lead to variable TRF in tumor cells. There is no direct relationship between telomeric repeat length and telomerase expression in tumors, because telomerase expression possibly leads to telomere repair (Kim et al. 1994). In this respect, our finding that 5/30 tumors showed elongation of telomeres is highly interesting. The literature on non-epithelial tumors supports these data. Thus, in gliomas, telomerase activity even leads to a considerable increase in telomeric repeat length (Morin 1990). Nürnberg et al. (1993) described telomere elongations in approximately 41.7% of the intracranial tumors investigated . In this respect, Mehle et al. (1996) suggested a telomerase-independent mechanism for TRF maintenance in renal cell tumors. Zakian (1997) discusses mechanisms other than telomerase activation for maintenance of telomeric lengths such as telomere-telomere recombination.

Considering the grading of the tumors, Odagiri et al. (1994) reported that the degree of telomeric repeat reduction correlated significantly with histological grade III in breast tumors. In contrast, we found only 2/9 GIII tumors with loss of telomere DNA. As telomeric lengths reflect the proliferative activity of a tumor, GIII tumors (see Table 1) are thought to display a considerable shortening of telomeres. Possibly GII and particularly GIII tumors express telomerase at higher levels or in phases of telomere shortening (Mehle 1994). Hiyama et al. (1995) described missing or reduced telomerase activity in early stages of stomach carcinomas. Similarly, Gupta et al. (1996) considered the requirement for telomerase activity to be a late event in malignant cell proliferation. In chronic lymphoid leukemia (usually characterized by limited cell proliferation), elevated telomerase activity was detected only in the late stage of the disease. In contrast to these findings, Hastie et al. (1990) reported an early onset of telomere shortening in prostate tissues and neoplastic gastric and colorectal lesions. Therefore, telomerase activation may occur at different clinical stages in different cancers. We believe that the diverging telomere repeat lengths of our tumor material are indicative of variable timing of telomerase reactivation. Diploid tumors more often show telomeric repeat reduction ($P = 0.04$) and, as a consequence, shorter TRF. The reason for this may relate to degraded chromosomal ends that are thought to be responsible for chromosomal instability (Vaziri and Benchimol 1996). Nevertheless, this question can be answered by directly measuring the telomeric lengths at the onset of telomerase expression.

Differences in DNA fingerprints are another consequence of genetic instability in tumors (Agurell et al. 1992; Werely et al. 1997). Such DNA fingerprint differences (36.7%) were found at high frequency in soft-tissue sarcomas. As there is no significant association between TRF and the occurrence of DNA fingerprint alterations, a causal relationship between these two parameters cannot be assumed. We observed DNA fingerprint differences at a higher frequency in local recurrences. It has to be verified whether DNA fingerprint instabilities and telomere shortening correlate with the potential of a soft-tissue sarcoma to develop recurrences or metastases. The question remains whether telomere reduction and/or telomerase activity correlate with specific tumor types. Special attention will be focussed on malignant schwannomas and leiomyosarcomas. A large number of soft-tissue sarcoma types should be investigated to allow general conclusions to be reached.

**Acknowledgement** The authors thank Mrs. Hiltraud Scharfenort for excellent technical assistance and Dr. Dagmar Franke for performing the statistical analyses. The work was supported by the Wilhelm-Sander-Stiftung, Munich, Germany (97.011.1)

## References

Agurell E, Li R, Rannug U, Norming U, Tribukait B, Ramel C (1992) Detection of DNA alterations in human bladder tumors by DNA fingerprint analyses. Cancer Genet Cytogenet 60:53–60

Allsop RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB (1992) Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci USA 89:10114–10118

Blackburn EH (1991) Structure and function of telomeres. Nature 350:569–573

Counter CM, Avilion AA, Lefeuvre CE, Stewart NG, Greider CW, Harley CB, Hirte H, Bacchetti S, Harley CB (1992) Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 11:1921–1929

Counter CM, Hirte H, Bacchetti S, Harley CB (1994) Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci USA 91:2900–2904

Counter CM, Gupta J, Harley CB, Leber B, Bacchetti S (1995) Telomerase activity in normal leukocytes and in hematologic malignancies. Blood 85:2315–2320

De Lange T (1994) Activation of telomerase in a human tumor. Proc Natl Acad Sci. USA 91:2882–2885

DeLange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM, Varmus HE (1990) Structure and variability of human chromosome ends. Mol cell Biol 10:518–527

Enzinger FM, Weiss SW (1995) Soft tissue tumors. Mosby, St. Louis, Baltimore, 3rd edn

Epplen JT (1992) Advances in electrophoresis. 5:59–112

Epplen JT, Ammer H, Epplen C, Kammerbauer C, Mitreiter R, Roewer L, Schwaiger M, Steimle V, Zischler H, Albert E, Anderas A, Beyermann B, Meyer W, Buitkamp J, Nanda I, Schmid M, Nürnberg P, Pena SDJ, Pöche H, Sprecher W, Schartl M, Weising K, Yassouridis A (1991) Oligonucleotide fingerprinting using simple repeat motifs: a convenient, ubquitously applicable method to detect hypervariability for multiple purposes. In: Burke T, Dolf G, Jeffreys AJ, Wolff R (eds) DNA Fingerprinting: approaches and applications. Birkhäuser, Basel

Gupta J, Han LP, Wang P, Gallie BL, Bacchetti S (1996) Development of retinoblastoma in the absence of telomerase activity. J Natl Cancer Inst 88:1152–1157

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during aging of human fibroblasts. Nature 345:458–460

Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC (1990) Telomere reduction in human colorectal carcinoma and with aging. Nature 346:866–868

Hiyama E, Hiyama K, Yokoyama T, Ichikawa T, Matsuura Y (1992) Length of telomeric repeats in neuroblastoma: correlation with prognostic and other biological characteristics. Jpn J Cancer 83:159–164

Hiyama E, Yokoyama T, Tatsumoto N, Hiyama K, Imamura Y, Murakami Y, Kodama T, Piatyszek MA, Shay JW, Matsuura Y (1995) Telomerase activity in gastric cancer. Cancer Res 55:3258–3262

Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyam T, Gazdar AF, Hiyama K, Piatyszek MA, Shay JW (1996) Telomerase activity in human breast tumors. J Natl Cancer Inst 88:116–122

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015

Mehle C, Ljungberg B, Roos G (1994) Telomere shortening in renal cell carcinoma. Cancer 54:236–241

Mehle C, Piatyszek MA, Ljungberg B, Shay JW, Roos G (1996) Telomerase activity in human renal cell carcinoma. Oncogene 13:161–166

Morin G (1990) The human telomere terminal transferase is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59:521–529

Nürnberg P, Thiel G, Weber F, Epplen JT (1993) Changes of telomere lengths in human intracranial tumors. Hum Genet 91:190–192

Odagiri E, Kanda N, Jibiki K, Demura R, Aikawa E, Demura H (1994) Reduction of telomeric length and c-*erB-2* gene amplification in human breast cancer, fibroadenoma, and gynecomastia. Cancer 73:2978–2984

Royle NJ (1996) Telomeres and disease. J Pathol 180:233–235

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning – a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Schwartz HS, Juliao SF, Sciadini MF, Miller LK, Butler MG (1995) Telomerase activity and oncogenesis in giant cell tumor of bone. Cancer 75:1094–1099

Vaziri H, Benchimol S (1996) From telomere loss to p53 induction and activation of a DNA-damage pathway at senescence: the telomere loss/DNA damage model of cell aging. Exp Gerontol 31:295–301

Werely J, Heyns F, Van Velden DJJ, Van Helden PD (1997) DNA fingerprint detection of somatic mutations in benign prostatic hyperplasia and prostatic adenocarcinoma. Genes Chromosomes Cancer 17:31–36

Zakian VA (1997) Life and cancer without telomerase. Cell 91:1–3