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Telomerase Activity in Skeletal Sarcomas

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Abstract

Background—Telomerase is a ribonucleoprotein that adds TTAGGG nucleotide repeats onto the ends of eukaryotic chromosomes to maintain telomere integrity. Somatic cells do not express telomerase and stop dividing when the chromosomal ends are shortened critically after many cell divisions. Immortal cell lines and cancer cells apparently have telomerase activity that contributes to an unlimited number of cell cycles. The purpose of our study is to investigate whether telomerase activity is expressed in primary malignant tumors of the skeletal system when compared to adjacent normal tissue.

Methods—Fresh tumor and normal tissue was collected from 14 patients (10 males, 4 females; age range, 8 to 76 years) and protein extraction performed. The tumors included seven osteosarcomas (three examined before and after chemotherapy), two chondrosarcomas, two spindle cell tumors, one hemangiopericytoma, one chordoma, and one adamantinoma. Telomerase activity was analyzed by using a highly sensitive polymerase chain reaction (PCR)–based assay (telomere repeat amplification protocol [TRAP]).

Results—Telomerase activity was found in 8 of 14 sarcoma patients (57%) using the TRAP assay. Compared to HeLa cell extract (positive control), telomerase activity in the tumor specimen ranged from 0 (in osteosarcoma) to 11.7% (in hemangiopericytoma). There was variation in the number of telomeric repeats generated by telomerase. At least five telomeric bands (e.g. 50, 56, 62, 68, 74 bp) in a ladder pattern had to be present before telomerase activity was considered positive in our analysis.

Conclusions—Telomerase activity may be an oncogenic sustaining event helping to maintain the transformed phenotype seen in malignant tumors of the bone. The degree of telomerase activity varies among skeletal malignancies, but was less than that observed in HeLa cells. The majority of osteosarcomas showed no telomerase activity.

Keywords

Telomerase activity; Skeletal sarcomas; Osteosarcomas; TRAP assay

Primary malignant tumors of the skeletal system are rare. About 2000 new skeletal sarcomas are reported annually in the United States.[1] Osteosarcoma is the most common primary

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malignancy of bone. Approximately 750 individuals develop conventional osteosarcoma each year in the United States.[2] Advances in medical imaging, chemotherapy, bioengineering, and surgical technique have improved the prognosis and functional outcome in the treatment of skeletal sarcomas.[3,4] Survival results have plateaued, however, as chemoresistant sarcomas predominate.[5] New antineoplastic drugs and tumoricidal concepts require investigation.

Telomeres are specialized structures at the ends of eukaryotic chromosomes containing unique (TTAGGG)n repeats. The average telomere length in humans is between 5 and 15 kilobases. These repeats protect chromosome ends against illegitimate recombination and may direct chromosome attachment to the nuclear membrane. Their structure and mode of synthesis allow for replication of the chromosome ends; however, loss of chromosome terminal sequences occurs with each round of replication. Previously, we demonstrated loss of telomere size in leukocytes of healthy individuals of advancing age (average 40 base pairs per year), with the greatest loss occurring in the younger subjects, those less than 20 years of age.[6] Reduction in telomere length has been observed in several malignancies, including colon, glioblastoma, leukemia, Wilms, breast, lung, and giant cell tumor of bone, as well as the in vitro senescence of human fibroblasts.[7–13]

Telomeres appear elongated in fetal tissues, and the size is more homogeneous than in older individuals, in whom telomeres are shorter and more heterogeneous in size.[14]

Telomere integrity is maintained by a specific telomere transferase (telomerase), a ribonucleoprotein enzyme. Telomerase contains an RNA component that has a template region complementary to the (TTAGGG)n repeats that permit the *de novo* synthesis of telomeric DNA at the chromosome ends. Telomerase counteracts molecular senescence or telomere loss and is most active in germ cells, which have significantly longer telomeres than do somatic cells, in which telomerase is inactive and telomere shortening occurs naturally. The reactivation of telomerase in malignant cells may allow for stabilization of the telomere and may be important for the attainment of immortality by cancer cells. Telomerase activity has been reported in tumor cells, initially in giant cell tumor of bone[15] and ovarian cancer.[16] Recently, a highly sensitive polymerase chain reaction (PCR)–based telomerase assay called the telomerase repeat amplification protocol (TRAP) method has been developed for telomerase detection.[17] Using this method, telomerase activity has been found in a wide variety of tumors, including neuroblastoma,[18] lung cancer,[19] colorectal cancer,[20] hepatocellular carcinoma,[21] gastric cancer,[22] breast cancer,[23] squamous cell carcinoma,[24] and retinoblastoma.[25] There is a paucity of telomerase data from skeletal sarcomas. In the present study, telomerase activity is assayed in several skeletal sarcomas and compared with their clinical outcome.

PATIENTS AND METHODS

Patients and Tissue Samples

A total of 14 patients (10 male, 4 female) with skeletal sarcomas (10 osteosarcomas—7 patients before chemotherapy [3 of whom were also studied after chemotherapy]; 2 chondrosarcomas, 2 spindle cell tumors, 1 chordoma, 1 adamantinoma and 1

hemangiopericytoma) were included in this study. Seventeen tumor samples measuring 1 cm^3 were obtained intraoperatively from the 14 patients after informed consent. The average age of the patients was 42.8 years (range, 8 to 76 years). Clinical characteristics and descriptions of the patients are shown in Table 1. The tumor specimens used in our analysis were carefully selected from biopsies or surgical resections. Only tumor from the core or middle of the tumor specimen was analyzed, to increase the likelihood that the tumor specimen contained neoplastic cells. Pathological documentation of all tumors was performed. Non-tumor tissue specimens (1 cm^3), usually muscle, also were collected from surrounding sites for telomerase analysis as a negative control. Immediately after collection the specimen was placed on wet ice, frozen in liquid nitrogen, and stored at −80°C. The time from collection of the tissue specimen to storage did not exceed 30 minutes. HeLa cells were used as telomerase-positive control cells and were obtained commercially (National Institute of General Medical Sciences, Human Genetic Mutant Cell Repository, Camden, NJ).

Telomerase Assay

The tissue specimens were partially thawed, weighed, and 100 mg used for protein extraction. The tissue specimen was thoroughly washed with PBS buffer, then transferred to homogenizer tubes containing 200 μl cold (4°C) CHAPS lysis buffer (10 mM tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM Benzamidine, 5 mM beta-mercaptoethanol, and 0.5% CHAPS, 10% glycerol) and dispersed by a motorized homogenizer (Kontes, Vineland, NJ) following the manufacturer's instructions. Disposable pestles were used for each specimen. The homogenized samples were incubated at 4°C for 10 minutes, after which the cell lysate was spun at 14,000 rpm at 4°C for 30 minutes. Protein concentration was determined by the Bradford Assay (BioRad Laboratories, Richmond, CA). The supernatants were then aliquoted and stored at −80°C.

Telomerase assay was undertaken using the Trap-eze Telomere Kit from Oncor, Inc. (Gaithersburg, MD) and following the manufacturer's protocols. Radioactive isotope (32P) was used for the PCR method. The telomerase products were amplified for 30 minutes at 30°C, then for 27 cycles at 94°C for 30 seconds and at 60°C for 30 seconds. 1.5 μL of PCR products were loaded onto an 18-cm electrophoresis apparatus using 12% polyacrylamide gel and were run for 2 hours and 30 minutes at 500 volts. The dried gel was exposed to a Phosphoimager Screen (Molecular Dynamics, Sunnyvale, CA) and analyzed by the use of the Phosphoimager of the radioactive telomeric fragments generated by the TRAP telomerase assay, following the manufacturer's protocol. Telomerase products were also heat inactivated at 95 °C for 10 minutes and treated with 1 μL of RNase (10 mg/mL) for 30 minutes. Heat and RNase individually destroyed telomerase activity in the samples studied. Negative telomerase activity was recorded after 7 days of exposure to the Phosphoimager Screen. Positive activity was recorded after detection of 5 telomeric bands in a 6-bp repeat ladder pattern (e.g., 50, 56, 62, 68, 74 bp) identified by the Phosphoimager and the presence of the positive internal control 36-bp band. Telomerase activity was quantified by detecting radioactivity from the telomeric band formation in a ladder fashion and compared relatively to a HeLa cell extract treated similarly for telomerase activity with a protein concentration of 0.0006 μg. Higher protein concentrations showed inhibitions of telomerase activity, whereas lower concentrations showed lower activity, which agrees with other investigators. The

optimal protein concentration for HeLa cells was 0.0006 μg, which was used for comparison with protein extracts from tumor cells. Tumor protein concentrations of 0.3 μg, 0.03μg, and 0.003 μg were chosen for comparison with the HeLa cell extracts. The level 0.03 μg was considered the optimal concentration for all tumors studied. On all occasions the protein samples were restudied, and the telomerase results were similar on the repeated measures. Therefore, the telomerase findings were reproducible with this methodology.

Histopathology

To determine if the number and percentage of tumor cells and nonneoplastic cells, mitotic activity, presence of necrosis, and amount and type of extracellular material influenced telomerase activity, histopathology of each specimen was recorded. An independent pathologic review was undertaken from representative hematoxylin and eosin–stained microscope slides, which reconfirmed the diagnosis. Histopathology was recorded from five representative microscopic high-power fields (40×; 1690 μm^2 surface area/each). The average and standard deviation of cells seen per high-power field and the percentage of neoplastic cells were calculated along with additional histopathology data (Table 2).

RESULTS

Seventeen tumor samples were obtained from 14 patients. The clinical data are displayed in Table 1, and the telomerase data from the skeletal sarcoma patients are shown in Table 3.

Three patients (9, 10, 11) had tumor specimens collected before and after chemotherapy. It is uncertain how neoplastic cells and telomerase respond to cytotoxic therapy. Telomerase activity was observed in patient 10 with osteosarcoma (after chemotherapy only) and patient 11 with osteosarcoma (before chemotherapy only). Specimens from patient 14 (spindle cell tumor) and patient 12 (osteosarcoma) were collected only after chemotherapy but did demonstrate telomerase activity.

Eight of the 14 patients (57%) studied showed telomerase activity. Three of the 10 osteosarcoma specimens from seven patients showed low telomerase activity (0.2%, 0.4%, and 0.5%) compared to the HeLa cell extract used as a positive telomerase control. Both spindle cell tumors, which are undifferentiated tumors compared to osteosarcomas, also had detectable but low (0.3%) telomerase activity compared with HeLa. Adamantinoma, chondrosarcoma, and hemangiopericytoma specimens showed telomerase activity of 3.1%, 3.9%, and 11.2%, respectively, compared with HeLa (Fig. 1). In five of the eight tumor cases, telomerase activity was also found in a 10-fold dilution (0.003-μg protein concentration) compared to a 0.03-μg concentration, but in decreased levels. In the tumor specimens with the 0.03-μg protein concentration, the telomerase activity ranged from 0.3% to 11.2% of activity seen in HeLa at a concentration of 0.0006 μg. In two telomerase-positive samples, activity was observed only in the 0.03-μg tumor protein concentration. In most telomerase-positive tumor specimens, weak to moderate telomeric band signals were detected by the Phosphoimager, and 9 to 11 bands were observed. Several tumors, such as adamantinoma and hemangiopericytoma, showed an intense 6-bp repeat telomeric band ladder pattern in the first six telomere bands (50, 56, 62, 68, 74, 80 bp).

Other tumors, e.g., osteosarcomas and chondrosarcomas, had 9 to 11 telomeric bands detected by the Phosphoimager but showed, on average, weaker band signals. In all subjects, normal tissue (e.g., muscle) was collected at the time of tumor removal and telomerase activity examined. No telomerase activity was seen in the normal tissue.

Cell density varied among the skeletal sarcomas studied as well as within the tumor subgroups. For example, patient 8 (osteosarcoma) showed 555 tumor cells per high-power field, and patient 10a (osteosarcoma) showed 109 tumor cells. Both were studied before chemotherapy. The highest telomerase activity was seen in patient 4 (hemangiopericytoma), with the largest number of tumor cells observed per microscope high-power field. However, the second, third, and fourth largest number of tumor cells observed per high-power field were in osteosarcoma patients with no detectable telomerase activity. In addition, the lowest number of tumor cells was found in patient 11b (osteosarcoma), who had been treated with chemotherapy.

There appeared to be no correlation with mitotic activity, grade, metastasis, presence of necrosis or extracellular material, and telomerase activity. For example, two of the three osteosarcoma patients with marked osteoid material (patients 10b and 11a) showed telomerase activity. Two of the three tumors with multinucleated cells (patients 1 and 14) seen per high-power field showed telomerase activity. Fourteen of the 17 tumors were classified as high grade, including all osteosarcomas. Seven of the eight tumors (patients 1, 4, 10b, 11a, 12, 13, and 14) with telomerase activity were classified as high grade. Adamantinoma, a low-grade skeletal sarcoma, demonstrated telomerase activity. Two of the patients (1 [chondrosarcoma] and 8 [osteosarcoma]) developed metastasis to the lungs, but only patient 1 showed telomerase activity.

There appeared to be no correlation between age and telomerase activity. Telomerase activity was seen in patients ranging from 17 to 61 years of age. The percentage of tumor cells seen per high-power field ranged from 41% in patient 11b (osteosarcoma after chemotherapy) to 98% in patient 4 (hemangiopericytoma). No telomerase activity was observed in patients with under 69% tumor cells in our study. The average percentage of tumor cells in those patients exhibiting telomerase activity was 87%.

To investigate for inhibition of the TRAP assay we examined different tumor protein concentrations (0.3 μg, 0.03 μg, and 0.003 μg). In most cases, inhibition was observed at 0.3-μg concentration, with a decreased intensity and number of telomeric bands recognized. An additional band located between the 36-bp internal control band and the 50-bp band was found in those specimens showing inhibition. For the telomerase activity to be considered positive, at least five telomeric bands (50, 56, 62, 68, 74 bp) were present. Therefore, we chose the tumor protein concentration of 0.03 μg to be optimal for all tumor specimen assays to compare with the telomerase positive controls (e.g., HeLa). A similar procedure for determining telomerase inhibition of the HeLa cell extract was also undertaken. The optimal protein concentration for HeLa cell extract was determined to be 0.0006 μg (Fig. 2).

DISCUSSION

Telomerase activity was present in most of the skeletal sarcomas, either before or after chemotherapy. We compared the tumor TRAP assay signal densitometrically with a HeLa cell extract of known protein concentration and expressed the telomerase activity in percentage compared with HeLa. The range of telomerase activity was studied in different tumor concentrations to determine whether telomerase was inhibited at higher than optimal concentrations. The tumor and HeLa extracts were treated separately with heat and RNase to further confirm the presence of telomerase activity, which is inactivated by either treatment. The number of Phosphoimager-detected telomeric repeat bands also was recorded and telomerase activity compared with the clinical characteristics of each patient.

The data suggest that skeletal sarcomas have a much lower telomerase activity using the TRAP protocol than do HeLa cells, which represent a highly malignant tumor cell line commercially available and used as a telomerase positive control in our study. Gupta et al.[25] reported no detectable telomerase activity from an osteosarcoma cell line. We found no detectable telomerase activity in the original or the repeated assays in five of the seven freshly harvested osteosarcoma specimens examined before chemotherapy. We also found no telomerase activity (data not shown) in a benign chondroblastoma from the left humerus of a 15-year-old female. Bryan et al.[26] also reported no telomerase activity in immortal cells derived from fibroblasts and suggested that in some tumor cells telomere maintenance may be related to non-telomerase factors. Our observation of low levels of telomerase activity in skeletal sarcoma specimens compared with HeLa supports our earlier reports of low levels of telomerase activity in chordomas[27] and in giant cell tumor of bone[15] using the S-100 cell extraction protocols reported by Counter et al.[28] and Morin[29] and not based on the TRAP assay. The similar reported telomerase activity levels using different assays are evidence confirming that each assay is correct.

Histopathologic review revealed no correlation between mitosis, grade, necrosis, or extracellular material found in comparison with the telomerase activity recorded. Although our sample size is relatively small, the number of tumor cells seen may influence the TRAP protocol results. Sarcomas with the highest tumor cell density (i.e., hemangiopericytoma) showed the highest telomerase activity. This phenomenon of increased number of tumor cells and increased telomerase activity has been observed previously in in vitro experiments.[17]

Chemotherapy also may influence telomerase activity. For example, no telomerase activity was found in a recurrent osteosarcoma following chemotherapy (patient 11b). Those sarcomas with a high number of tumor cells (e.g., osteosarcoma patients 8, 9a, 9b) but with no telomerase activity may reflect genetic abnormalities. These genetic derangements could affect telomerase gene expression or its translation and not allow telomerase production. Telomerase activation may be passively co-selected as tumors develop or following the administration of neoadjuvant chemotherapy.

In our study we used a highly sensitive PCR-based telomeric repeat amplification protocol (TRAP) following the manufacturer’s guidelines, which allow for telomerase detection in

cell extracts (Oncor Inc., Gaithersburg, MD). Several potential problems that may influence the qualitative and quantitative results of telomerase detection have been described.[30,31] For instance, in some tissues, Taq polymerase inhibitors may be present, giving false-negative results. In addition, the assays used in the past were non-linear, which made the relative telomerase expression difficult to interpret in some tumors. With modification of the TRAP protocol and recognition of factors influencing telomerase activity (e.g., inhibition), the telomeric repeat amplification protocol has been much improved,[32] with more reproducible results, and successfully applied in our study.

We conclude that eight of the 17 skeletal sarcoma samples demonstrated telomerase activity. Because telomerase activity was found in 57% of our patients, levels of activity may reflect the different cell populations rather than the overall number of telomerase positive cells.[19] Identifying telomerase activity in skeletal sarcomas is of interest, because efforts currently are underway to develop telomerase inhibitors to serve in multi-agent chemotherapy protocols,[33] and potentially to augment the treatment strategy for sarcomas. It appears that skeletal sarcoma cells have other ways of maintaining stability at the ends of their chromosomes. Telomerase may be helpful, but is not essential, for skeletal sarcoma oncogenesis.

Acknowledgments

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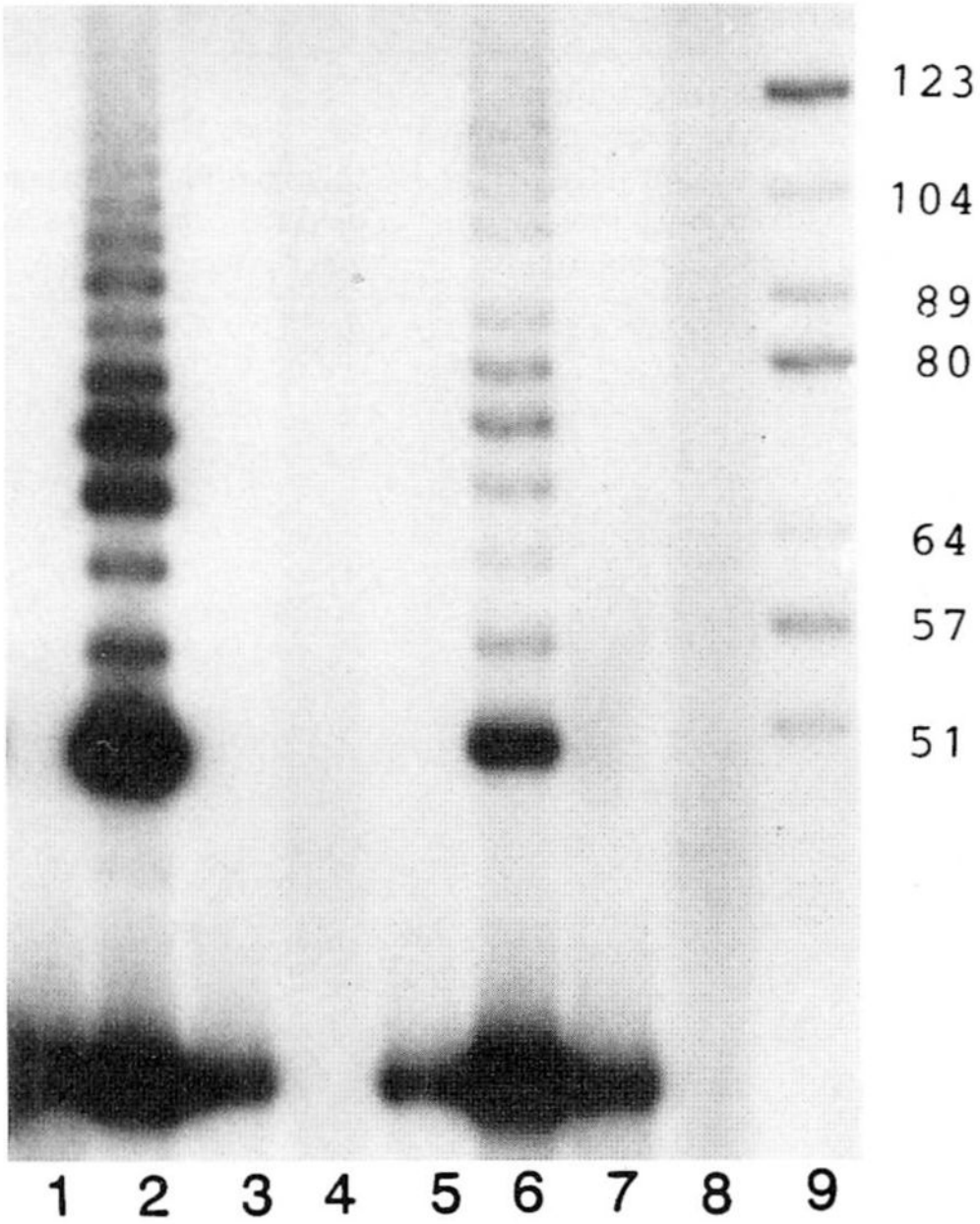

FIG. 1.

Polyacrylamide gel (12%) showing evidence of telomerase activity from protein extract of tumor cells from skeletal sarcoma patients and HeLa, a positive control cell line. *Lane 1:* PCR reaction without protein extract. *Lane 2:* Hemangiopericytoma (0.03 μg protein). *Lane 3:* Hemangiopericytoma (0.03 μg protein)—heat treated. *Lane 4.* Hemangiopericytoma (0.03 μg protein)—RNase treated. *Lane 5:* muscle (0.03 μg protein). *Lane 6:* HeLa (0.0006 μg protein). *Lane 7:* HeLa (0.0006 μg protein)—heat treated. *Lane 8:* HeLa (0.0006 μg protein)—RNase treated. *Lane 9:* DNA marker 5 (Boehringer Mannheim, Indianapolis, IN). The numbers represent DNA marker fragment sizes in base pairs. The internal standard (36 bp) is shown at the bottom of the figure.

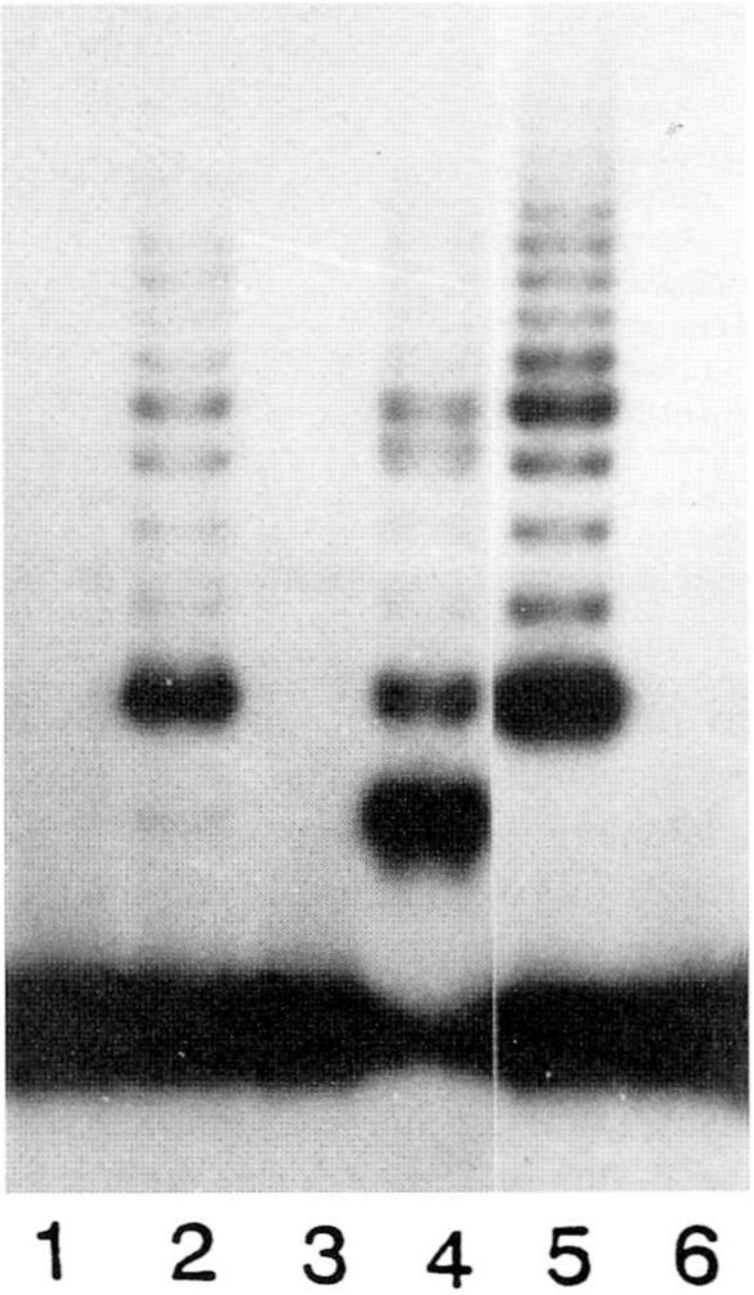

FIG. 2.

Polyacrylamide gel (12%) showing telomerase activity and inhibition in chondrosarcoma (patient 1). HeLa is a positive control cell line. *Lane 1:* Chondrosarcoma (0.03 μg protein)—heat treated. *Lane 2:* Chondrosarcoma (0.03 μg protein). *Lane 3:* Chondrosarcoma (0.3 μg protein)—heat treated. *Lane 4:* Chondrosarcoma (0.3 μg protein) showing inhibition with an additional band between 36 and 50 bp. *Lane 5:* HeLa (0.0006 μg protein). *Lane 6:* HeLa (0.0006 μg protein)—heat treated.

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TABLE 1

Clinical data for skeletal sarcoma patients

Patient	Tumor specimen	Age (yr)	Sex	Location and surgical procedure	Local recurrence of tumor (months followed)	Metastasis	Histologic grading of the tumor*	Telomerase activity†
1	Chondrosarcoma	56	F	L femur resection	No (died, 11)	Lungs	High grade	Yes
2	Chondrosarcoma	76	F	L humerus resection	No (10)	None	Low grade	No
3	Chordoma	66	M	Sacral resection	Yes (14)	None	Low grade	No
4	Hemangiopericytoma	50	M	L humerus resection	No (13)	None	High grade	Yes
5	Adamantinoma	43	M	L fibula resection	No (10)	None	Low grade	Yes
6	Osteosarcoma	27	M	R tibia resection	No (10)	Lungs	High grade	No
7	Osteosarcoma	16	M	R femur resection	No (10)	None	High grade	No
8	Osteosarcoma	8	M	L tibia biopsy	No (19)	Lungs	High grade	No
9a	Osteosarcoma (before chemotherapy)	60	M	R femur biopsy	No (9)	None	High grade	No
9b	Osteosarcoma (after chemotherapy)			R femur resection	No (7)	None	High grade	No
10a	Osteosarcoma (before chemotherapy)	50	F	R femur biopsy	No (9)	Lungs	High grade	No
10b	Osteosarcoma (after chemotherapy)			R femur resection	No (7)	Lungs	High grade	Yes
11a	Osteosarcoma (before chemotherapy)	20	F	L femur biopsy	No (10)	Lungs	High grade	Yes
11b	Osteosarcoma (after chemotherapy)			L femur resection	No (8)	Bone	High grade	No
12	Osteosarcoma (after chemotherapy)	17	M	R femur resection	Yes (died, 19)	Lungs	High grade	Yes
13	Spindle cell tumor	39	M	L metatarsal resection	No (10)	None	High grade	Yes
14	Spindle cell tumor (after chemotherapy)	61	M	L femur resection	No (12)	None	High grade	Yes

*High grade = poorly differentiated tumor, high mitotic rate, likely to metastasize; low grade = well differentiated tumor, low mitotic rate, likely to recur locally.

†Telomerase activity was present when at least five telomere repeat bands (e.g., 50, 56, 62, 68, 74 bp) were detected by the Phoshoimager (Molecular Dynamics, Sunnyvale, CA).

TABLE 2

Pathology data for skeletal sarcoma patients

Patient	Tumor specimen	Histologic grading of the tumor*	Tumor cells† (average/SD)	Normal cells† (average/SD)	Tumor cells (%)	Mitosis‡	Necrosis	Comment	Telomerase activity§
1	Chondrosarcoma	High grade	312/22	21/20	94	Low	No	10% multinucleated cells present	Yes
2	Chondrosarcoma	Low grade	63/32	10/21	86	Low	No	Marked chondroid present	No
3	Chordoma	Low grade	240/44	24/37	91	Low	No	Marked chondroid present	No
4	Hemangiopericytoma	High grade	881/126	28/26	98	Low	No	Very vascular	Yes
5	Adamantinoma	Low grade	135/17	60/25	69	Low	Mild	None	Yes
6	Osteosarcoma	High grade	202/57	17/18	92	Intermediate	No	Moderate chondroid, osteoid present	No
7	Osteosarcoma	High grade	288/28	54/25	84	Low	No	Marked osteoid, bone present	No
8	Osteosarcoma	High grade	555/50	57/26	91	Low	No	10% multinucleated, mild osteoid present	No
9a	Osteosarcoma (before chemotherapy)	High grade	532/82	33/12	94	Low	Marked	None	No
9b	Osteosarcoma (after chemotherapy)	High grade	615/73	56/27	92	High	Mild	Mild osteoid present	No
10a	Osteosarcoma (before chemotherapy)	High grade	109/25	48/18	69	Low	Moderate	Moderate chondroid present	No
10b	Osteosarcoma (after chemotherapy)	High grade	309/29	47/19	87	Low	Moderate	Marked osteoid, chondroid, bone present	Yes
11a	Osteosarcoma (before chemotherapy)	High grade	527/30	33/27	94	Intermediate	No	Marked osteoid and bone present	Yes
11b	Osteosarcoma (after chemotherapy)	High grade	41/44	60/12	41	Low	Marked	Mild bone present	No
12	Osteosarcoma	High grade	219/64	24/21	90	Low	Marked	None	Yes
13	Spindle cell tumor	High grade	464/34	96/18	83	Intermediate	No	Dense fibrous background	Yes
14	Spindle cell tumor	High grade	333/19	84/9	80	Low	No	10% multinucleated, marked osteoid present	Yes

*High grade = poorly differentiated rumor, high mitotic rate, likely to metastasize; low grade = well differentiated tumor, low mitotic rate, likely to recur locally.

†Average number +/− standard deviation of cells based on five representative high power fields (40×, 1694 μm^2) analyzed from hematoxylin and eosin–stained microscope slides of each tumor specimen.

‡Mitosis = low (0–1 mitotic figures seen per high-power field [40×]); intermediate (2–5 mitotic figures); high (>5 mitotic figures).

§Telomerase activity was present when at least five telomere repeat bands (e.g., 50, 56, 62, 68, 74 bp) were detected by the Phoshoimager (Molecular Dynamics, Sunnyvale, CA).

TABLE 3

Telomerase data for skeletal sarcoma patients

Patient	Tumor specimen	Telomerase activity* in 0.03 μg tumor protein concentration	Telomerase activity in 0.003-μg tumor protein concentration	Telomerase activity† (% activity compared with positive control [HeLa cells; (0.0006-μg protein concentration)]	Number Phosphoimager detected bands at 0.03-μg tumor protein concentration
1	Chondrosarcoma	Yes	Yes	3.9	11
2	Chondrosarcoma	No	No	—	—
3	Chordoma	No	No	—	—
4	Hemangiopericytoma	Yes	Yes	11.7	8
5	Adamantinoma	Yes	Yes	3.1	7
6	Osteosarcoma	No	No	—	—
7	Osteosarcoma	No	No	—	—
8	Osteosarcoma	No	No	—	—
9a	Osteosarcoma (before chemotherapy)	No	No	—	—
9b	Osteosarcoma (after chemotherapy)	No	No	—	—
10a	Osteosarcoma (before chemotherapy)	No	No	—	—
10b	Osteosarcoma (after chemotherapy)	Yes	No	0.4	9
11a	Osteosarcoma (before chemotherapy)	Yes	No	0.5	5
11b	Osteosarcoma (after chemotherapy)	No	No	—	—
12	Osteosarcoma (after chemotherapy)	Yes	Yes	0.2	9
13	Spindle cell tumor	Yes	No	0.3	10
14	Spindle cell tumor (after chemotherapy)	Yes	Yes	0.3	7

*Optimal tumor protein concentration for detection of telomerase activity was 0.03 μg; higher concentrations inhibited telomerase activity in most tumor specimens.

†Telomerase activity was considered negative after 7 days exposure to the Phosphoimager (Molecular Dynamics, Sunnyvale, CA).