Telomerase Activity and Expression of hTRT and hTR in Gastrointestinal Stromal Tumors in Comparison with Extragastrointestinal Sarcomas

Thomas Günther, Regine Schneider-Stock, Carslen Häckel, Matthias Pross, Hans-Ulrich Schulz, Hans Lippert, and Albert Roessner

Departments of Pathology [T. G., R. S.-S., C. H., A. R.] and General Surgery [M. P., H. U. S., H. L.], Otto-von-Guericke University, 39120 Magdeburg, Germany

ABSTRACT

Stromal tumors of the gut (GISTs) have rarely been analyzed for genetic alterations. This study aimed at determining telomerase activity and the expression of the telomerase subunits human telomerase reverse transcriptase (hTRT) and human telomerase RNA (hTR) in GISTs and extragastrointestinal neurogenic or myogenic sarcomas. Telomerase activity was investigated using the telomeric repeat amplification protocol assay in 21 GISTs, recurrences and liver metastases from 16 patients, and in 22 leiomyosarcomas and 21 malignant peripheral nerve sheath tumors (MPNSTs), which served as reference tumors. Expression of hTRT and hTR mRNA was investigated using reverse transcription-PCR. Thirteen GISTs were localized in the stomach and three in the small intestine. Two tumors were benign. In one case, the biological behavior was uncertain. In 67% of GISTs, high telomerase activity was found, whereas high activity was noted in only 18% of leiomyosarcomas and in 48% of MPNSTs. There was no activity in two benign and two malignant GISTs. In one malignant tumor of the small intestine, the primary tumor showed no activity at first but a marked activity in its recurrence. In the tumor with uncertain behavior, telomerase activity and hTRT expression were only weak. In all GISTs showing telomerase activity, the catalytic subunit hTRT was expressed. All GISTs and extragastrointestinal sarcomas expressed hTR. In comparison with leiomyosarcomas and MPNSTs, malignant GISTs showed a higher telomerase activity, which, however, was not seen in benign GISTs. It is possible that telomerase activity occurs during the progression of malignant GISTs. There was a correlation between telomerase activity and the expression of hTRT.

INTRODUCTION

Stromal tumors of the gut (GISTs) are a rare heterogeneous group of neoplasms with unclear histogenesis, and although they may bear a strong histomorphological resemblance to extragastrointestinal soft tissue tumors of neurogenic or myogenic origin, GISTs do not show a clear ultrastructural and/or immunohistological differentiation.

Because of the biological behavior of GISTs, both pathologists and surgeons are faced with problems related to diagnostics and therapy. Golden and Stout (1) were the first to describe mesenchymal tumors of the gut. Because of the cellular characteristics of these tumors Stout (2) interpreted them as myogenic neoplasms and introduced the term “leiomyoblastomas” for those tumors composed of epithelioid cells (3). In 1969, electron microscopy revealed that not all tumors described by Stout (2) showed myogenic characteristics (4). By contrast, there were indications of Schwann cells, which are suggestive of a neurogenic origin (5). About 20 years later, with the help of immunohistology, neurogenic (6) as well as myogenic (7) markers could be found.

Partially, the marker profile depends on the localization of these tumors in the gut (8, 9). The frequent observation of a positive immunohistological reaction with the CD34 antibody, with the majority of GISTs being positive for this hematopoietic progenitor cell antigen, allowed the conclusion that these tumors are neoplasms originating from primitive mesenchymal cells (10). Recent investigations, which meanwhile have been corroborated by molecular genetic investigations, show that GISTs have common features with the interstitial cell of Cajal, the so-called pacemaker cells of the gut (11, 12). In these cells, CD117 is expressed as the product of the c-kit proto-oncogene and plays a role in the development of this cell type (13). Sarlomo-Rikala et al. (14) pointed out that the CD117 antibody is a useful marker for diagnosing GISTs and for separating them from true leiomyomas and neurogenic tumors. To date, only a few studies have investigated molecular genetic markers for the characterization of the biological behavior of GISTs.

The importance of telomerase has already been validated concerning the expression and progression of numerous malignant epithelial tumors (15–18). This enzyme regulates the de

Received 9/22/99; revised 1/28/00; accepted 2/1/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The research work was supported by the Grant 10-1501-Schn2 from the Dr. Mildred-Scheel-Stiftung, Bonn, Germany, and by Grant 97.011.2 from the Wilhelm-Sander-Stiftung, Munich, Germany.

2 To whom requests for reprints should be addressed, at Department of Pathology, Otto-von-Guericke University, Leipziger Strasse 44, 39120 Magdeburg, Germany. Phone: 49-391-67-13182; Fax: 49-391-67-15818; E-mail: Thomas.Guenther@Medizin.Uni-Magdeburg.de.

3 The abbreviations used are: GIST, gastrointestinal stromal tumor; hTR, human telomerase RNA; hTRT, human telomerase reverse transcriptase; MPNST, malignant peripheral nerve sheath tumor; NSE, neuron-specific enolase; TRAP, telomeric repeat amplification protocol; RT-PCR, reverse transcription-PCR; β2-M, β2-microglobulin.
Telomerase Activity and hTRT and hTR in GISTs

The extragastrointestinal sarcomas were graded according to the "cutoff point" of the mitotic activity between benign and malignant. The survival of particularly strongly proliferating tissue, such as malignant tumors, is very closely associated with the activation of this enzyme. Histologically, the biological behavior of the GISTs correlates with the survival of particularly strongly proliferating tissue, such as malignant tumors (19).

The aim of our study was to investigate telomerase activity and the expression of the telomerase subunits: hTRT and hTR, making a comparison with extragastrointestinal, myogenic, or neurogenic sarcomas. Moreover, we addressed the issue of whether histologically, the biological behavior of the GISTs correlates with telomerase activity, and which clinicopathological factors show an association with the activation of this enzyme.

PATIENTS AND METHODS

Tissue. Both telomerase activity and the expression of hTRT and hTR were investigated in 21 GISTs, recurrences, and liver metastases from 16 patients. Two GISTs incidentally observed at an autopsy were classified as benign. Twenty-two leiomyosarcomas and 21 MPNSTs served as extragastrointestinal reference tumors.

Methods. The tumor specimens were routinely processed at the Department of Pathology, Otto-von-Guericke University. The tumors were classified according to the grading system proposed by Lewin (22). According to Amin et al. (23), the "cutoff point" of the mitotic activity between benign and malignant was determined as 5 mitoses per 50 high power field. The extragastrointestinal sarcomas were graded according to the system proposed by Trojani et al. (24). In addition to routine preparation, further representative tumor materials from GISTs and the extragastrointestinal sarcomas were prepared, snap-frozen in liquid nitrogen, and stored at −80°C. To estimate the percentage of tumor cells in H&E slides, tumor tissue corresponding to all snap-frozen tumor samples was taken. To prevent false-positive results, we used only those cases in which at least 80% of tumor cells were seen. A dilutional effect on the DNA genetic analysis could thus be excluded. Because the small GISTs could be ciliated very easily from the adjacent tissue, there was no need for a microscopic dissection. In the case of large tumors, tissue without necroses was obtained from the center of the tumor.

Immunohistology. The immunohistochemical reactions were performed on formalin-fixed and paraffin-embedded tissue sections using the ABC (avidin-biotin-complex) method with either peroxidase or alkaline phosphatase. Paraffin sections were deparaffinated, rehydrated, and exposed to the primary antibodies. The antibodies for NSE (Vector, Burlingame, CA; dilution, 1:20) were diluted in a buffer containing 50 ml of RPMI 1640 (Life Technologies, Inc., Eggenstein, Germany), 450 ml of distilled water, 50 ml of fetal bovine serum (Life Technologies, Inc., Eggenstein, Germany), and 0.5 g of sodium azide (Sigma Chemical Co., Deisenhofen, Germany; pH, 7.4–7.6). The other antibodies were diluted in a commercially available dilution buffer (Ventana Medical Systems, Tucson, AZ).

Stainings for Desmin (Immunotech; dilution, 1:150), SM-Action (Immunotech; dilution, 1:150), S-100 (Dako, Hamburg, Germany; dilution, 1:500) and CD 34 (Biogenex, Hamburg, Germany; dilution, 1:20) were done automatically on the Ventana Nexes Immunostainer (Ventana Medical Systems) with peroxidase as detection system, whereas NSE and CD117 were stained manually at 37°C. Detection was done either by Ultra-tech-peroxidase (CD117) or Vectastain-alkaline phosphatase (NSE). For alkaline phosphatase, the following substrate solution was used and incubated for 25 min with gentle agitation: 4 ml of Tris buffer, 16 ml of propandiol, 0.025 g of levamisole, 0.125 g of newfuchsin, 0.3 ml of sodium nitrite (4%), 0.035 g of naphthol-as-bi, and 0.4 ml of dimethylformamide (all Sigma) at pH 8.7. For peroxidase, a commercially available diaminobenzidine solution was used (Immunotech), following the manufacturer’s instructions. All substrate reactions were performed at room temperature. The sections were counterstained with Mayer’s hematoxylin and embedded in gelatin.

 Determination of Telomerase Activity. Frozen tumor tissue was homogenized in 200 μl of 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate lysis buffer (telomerase extraction buffer) on ice (25). After 30 min of incubation on ice, the lysate was centrifuged at 16,000 × g, and the supernatant was snap frozen in liquid nitrogen and stored at −80°C. Protein concentration was measured by the Bradford assay (Bio-Rad Laboratories, Richmond, CA). To exclude variations in telomerase activity resulting from Taq polymerase inhibitors present in the tissue extract, we tested a series of dilutions (4, 0.4, and 0.2 μg/μl).

Telomerase activity was determined by using the ONCOR TRAP-eze telomerase detection kit (Oncor; Ref. 26). The following control reactions were done: (a) amplification of a 36-bp internal standard (provided in the kit) to identify false-negative samples containing Taq polymerase inhibitors; (b) a heat-inactivated control as a negative control; (c) a negative control without addition of a protein extract to exclude PCR-contamination; and (d) a TSR8-control, which should yield five to six signals (provided in the kit).

The TRAP assay was considered valid when all of the controls showed the expected values. To minimize the possibility of false-negative results because of tissue degradation, the suitability of the RNA in the protein extract was tested by RT-PCR amplification of a 495-bp fragment of the human β-actin gene with the primers 5'-CATGCCATCTCCTGCTCT-3' and 5'-CACGAGTACTTGCCTCAGGAGG-3'.

Cy5-labeled PCR products were electrophoresed on 0.5-mm thick polyacrylamide gels containing 7 m urea (Ready Mix; Pharmacia Biotech) on an automated sequencer (ALF Express; Pharmacia Biotech) and detected as a ladder of 6-base signals (provided in the kit).

Interpretation of Data. The definition of a genuine telomerase signal was any measurable signal that was greater than the signal level measured from the same area in the corresponding heat-inactivated control reaction. A value was considered too low if the signal strength of telomerase-positive reaction was equal or less than the signal strength of its corresponding heat

novo synthesis of the telomeres, the ends of eukaryotic chromosomes, protecting them from loss, fusion, and degradation (19, 20). Telomeres consist of thousands of copies of the single hexanucleotide repeat TTAGGG.

With each cell division, the chromosomal ends undergo shortening as long as they have reached a critical length, leading to apoptosis (21). Hence, telomeres virtually constitute one of the life-maintaining mechanisms of the cell. If the chromosomal ends are renewed by activating telomerase, the cell will be immortal. This is thought to be one mechanism that guarantees the survival of particularly strongly proliferating tissue, such as malignant tumors (19).

The tumors were classified according to the grading system proposed by Lewin (22). According to Amin et al. (23), the “cutoff point” of the mitotic activity between benign and malignant was determined as 5 mitoses per 50 high power field. The extragastrointestinal sarcomas were graded according to the system proposed by Trojani et al. (24). In addition to routine preparation, further representative tumor materials from GISTs and the extragastrointestinal sarcomas were prepared, snap-frozen in liquid nitrogen, and stored at −80°C. To estimate the percentage of tumor cells in H&E slides, tumor tissue corresponding to all snap-frozen tumor samples was taken. To prevent false-positive results, we used only those cases in which at least 80% of tumor cells were seen. A dilutional effect on the DNA genetic analysis could thus be excluded. Because the small GISTs could be ciliated very easily from the adjacent tissue, there was no need for a microscopic dissection. In the case of large tumors, tissue without necroses was obtained from the center of the tumor.
reaction. A value was considered positive if the signal strength was at least twice the SD of its corresponding heat-treated reaction. The TRAPEze telomerase kit uses a modified reverse primer sequence that allows the estimation of telomerase processivity. An extract was considered negative for telomerase activity when the 36-bp band was revealed and no discernable ladder pattern was found. Telomerase activity was interpreted as weak when only the 50-bp band was seen. Telomerase activity was considered highly positive when the 36-bp internal control band and a ladder of PCR products with 6-base increments were visible.

Determination of hTRT and hTR mRNA Expression.
Total RNA was isolated by using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) and treated with DNase (Life Technologies, Inc., Gaithersburg, MD). Reverse transcription was made according to the manufacturer’s instructions (Promega Corp., Madison, WI). RT-PCR conditions of hTRT and hTR were described by Schneider-Stock et al. (28).

hTRT primers were: sense, 5’-CGGAAGAGTGCTGGGAGC AA-3’; and antisense, 5’-GGATGGAGC GGAGCTGGGA-3’. hTR primers were: sense, 5’-CTTAC ACCCTAAGCTTAGAAGGGCGTAG-3’; and antisense, 5’-GTTTGCTCTAGAATGAACGGTGGAAG-3’.

Amplification was performed in an automated thermal cycler (Multicycler PTC 200; MJ Research, Watertown, MA) for 31 cycles (hTRT) and 28 cycles (hTR), respectively. Cycling conditions were: 94°C for 45 s, 60°C for 45 s, and 72°C for 90 s. HTRT and hTR mRNA expressions were determined using a semiquantitative RT-PCR assay.

β2-M mRNA was amplified to normalize equal amounts of RNA template using the primers and PCR conditions described in one of our previous studies (29). A negative control reaction without cDNA was included in each experiment. PCR products were resolved on ultrathin native polyacrylamide gels cross-linked with piperazine diacrylamide, baked on GelBond Pag (FMC, Rockland, ME), and visualized by silver staining (30). Bands were directly quantified with the use of laser densitometry (VDS; Pharmacia Biotech). Expression was evaluated by estimating the intensity ratio between the hTRT or hTR signal and the corresponding β2-M signal. Normal placenta tissue of an adult woman was used as a negative control. The expression level was defined as normal in the telomerase-negative placenta tissue. In comparison with the placenta, 3-fold or more intensified signals were assessed as increased expression. The expressions of hTRT and hTR were reported as absent or present for the purpose of this study. Different expression levels were not

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Gender</th>
<th>Localization</th>
<th>Size (cm)</th>
<th>Metastases</th>
<th>Growth mitoses in 50 HPF a</th>
<th>Grade</th>
<th>CD34</th>
<th>CD117</th>
<th>Desmin</th>
<th>sm-actin</th>
<th>S100</th>
<th>NSE</th>
<th>Follow-up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. H, P</td>
<td>54</td>
<td>M</td>
<td>Stomach</td>
<td>24</td>
<td>Liver</td>
<td>Spindle cell 100</td>
<td>Malignant</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>DOD b</td>
</tr>
<tr>
<td>2. K, K</td>
<td>58</td>
<td>M</td>
<td>Stomach</td>
<td>5</td>
<td>Liver</td>
<td>Spindle cell 2</td>
<td>Borderline</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>3. Z, D</td>
<td>60</td>
<td>M</td>
<td>Stomach</td>
<td>30</td>
<td>Liver</td>
<td>Epithelioid &gt;100</td>
<td>Malignant</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>4. Z, E</td>
<td>64</td>
<td>M</td>
<td>Stomach</td>
<td>13</td>
<td>Liver</td>
<td>Epithelioid 14</td>
<td>Malignant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>5. B, T</td>
<td>37</td>
<td>M</td>
<td>Stomach</td>
<td>12</td>
<td>Liver</td>
<td>Epithelioid 100</td>
<td>Malignant</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>6. S, G</td>
<td>70</td>
<td>M</td>
<td>Stomach</td>
<td>13</td>
<td>Liver</td>
<td>Epithelioid &gt;100</td>
<td>Malignant</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>23</td>
</tr>
<tr>
<td>7. T, R</td>
<td>64</td>
<td>M</td>
<td>Stomach</td>
<td>5.3</td>
<td>Liver</td>
<td>Epithelioid 19</td>
<td>Malignant</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>8. M, H</td>
<td>67</td>
<td>M</td>
<td>Stomach</td>
<td>7.5</td>
<td>Liver</td>
<td>Epithelioid 11</td>
<td>Malignant</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>9. V, I</td>
<td>53</td>
<td>F</td>
<td>Stomach</td>
<td>11</td>
<td>Liver</td>
<td>Epithelioid 40</td>
<td>Malignant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>DOD</td>
</tr>
<tr>
<td>10. J, D</td>
<td>47</td>
<td>F</td>
<td>Duodenum</td>
<td>5.6</td>
<td>Liver</td>
<td>Spindle cell 16</td>
<td>Malignant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>11. M, E</td>
<td>72</td>
<td>F</td>
<td>Ileum</td>
<td>11</td>
<td>Liver</td>
<td>Epithelioid 3</td>
<td>Malignant</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>15 years</td>
</tr>
<tr>
<td>12. M, D</td>
<td>64</td>
<td>F</td>
<td>Jejunum</td>
<td>10</td>
<td>Liver</td>
<td>Spindle cell 5</td>
<td>Malignant</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>13. S, R</td>
<td>9</td>
<td>F</td>
<td>Stomach</td>
<td>6.5</td>
<td>Liver</td>
<td>Epithelioid 12</td>
<td>Malignant</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>14. D, R</td>
<td>61</td>
<td>M</td>
<td>Stomach</td>
<td>17</td>
<td>Liver</td>
<td>Epithelioid 15</td>
<td>Malignant</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>15. G, L</td>
<td>84</td>
<td>F</td>
<td>Stomach</td>
<td>1.8</td>
<td>Liver</td>
<td>Spindle cell 0</td>
<td>Benign</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>Incidental finding</td>
</tr>
<tr>
<td>16. K, H</td>
<td>85</td>
<td>F</td>
<td>Stomach</td>
<td>2.5</td>
<td>Liver</td>
<td>Spindle cell 0</td>
<td>Benign</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Incidental finding</td>
</tr>
</tbody>
</table>

a HPF, high power field.

b DOD, died of disease.
classified. RNA was reverse transcribed twice, and each RT-PCR was done at least twice to ensure consistency. The NCI-H23 lung carcinoma cell line and human 293 cells were used as positive controls with known elevated hTRT mRNA expression and hTR mRNA expression (31). All cell lines were obtained from the American Type Culture Collection (Rockville, MD).

**Statistical Evaluation.** The significance of associations between genetic and immunohistological findings and clinicopathological factors was evaluated statistically by Fisher’s exact test (two tailed) and the χ² test according to Pearson using the SAS statistical software system (SAS Institute, Inc., Cary, NC). A probability of \( P < 0.05 \) was considered significant.

**RESULTS**

**Clinicopathological Findings.** The clinicopathological data of all patients with GISTs are summarized in Table 1. In 13 cases, the primary GISTs were localized in the stomach, and in the small intestine in three. The mean age of the patients was 59.3 years (9–85 years).

Of all GISTs localized in the stomach, only two tumors were defined as benign. The tumor diameters of these tumors were 1.8 and 2.5 cm, whereas the mean diameter of the malignant GISTs in the stomach was 13.0 cm (range, 4–30 cm). One gastric stromal tumor showed an uncertain malignant potential and was classified as borderline tumor. The diameter of this tumor was 5.0 cm. All three stromal tumors in the small intestine were classified as malignant, their diameters being 5.6, 10.0, and 11.0 cm.

Almost all GISTs, independent of their localization, showed a strong immunoreaction with the CD34 and CD117 antibodies. Only two gastric stromal tumors (15.4%) expressed myogenic markers (sm-actin and/or desmin), whereas nine tumors (69.2%) showed a neurogenic marker profile (S100-protein and/or NSE) at the same time.

In all three intestinal stromal tumors, neurogenic as well as myogenic markers were expressed. The CD34 and CD117 antibody reactions were negative in these tumors.

**Telomerase Activity in GISTs Compared with Extra-gastrointestinal Sarcomas.** In 81% (17 of 21) of the malignant GISTs, in their recurrences and liver metastases, telomerase activity was found. Sixty-seven % showed high activity (Fig. 1). The gastric stromal tumor with an uncertain malignant potential expressed a high level of telomerase activity (Fig. 1).

**Fig. 1** Nonradioactive TRAP assay in GISTs. Most tumors (samples 1–4) express a high telomerase activity level. The 36-bp internal control and a ladder pattern of more than six increments are visible (4 μg/μl protein extract). Sample 5 shows no telomerase activity; only the 36-bp internal control band is visible.

**Fig. 2** Nonradioactive TRAP assay showing leiomyosarcomas (4 μg/μl protein extract) with low (samples 1 and 2) and high telomerase activity (sample 3). Tumors with low telomerase activity show only the 50-bp increment, whereas the high-positive tumor shows a ladder-like pattern of PCR products.
potential (case 2) showed only low telomerase activity. There was no activity in two benign and two malignant tumors. In one malignant stromal tumor of the small intestine, the primary tumor showed no activity at first but displayed a marked activity in its recurrence (case 12).

In 68% of all leiomyosarcomas (15 of 22) and 71% of all MPNSTs (15/21), telomerase activity was noted (Fig. 2); however, high activity was found in only 18% of leiomyosarcomas (4 of 22) and in 48% of MPNSTs (10 of 21). Although there was no significant difference in the enzyme activation in all tumor groups investigated in this study ($P = 0.683$), GISTs showed a remarkable higher level of telomerase activity ($P = 0.024$).

There was no association between telomerase activity and tumor grade and localization of the extragastrointestinal sarcomas. Furthermore, an association of telomerase activity with gender and the age of the patients with GIST or extragastrointestinal sarcomas was not observed.

**Expression of the Telomerase Subunits in GISTs and Extragastrointestinal Sarcomas.** Despite tumor grade, localization, and the clinical data, all GISTs and extragastrointestinal sarcomas showed an expression of hTR, even if there was no telomerase activity.

On the other hand, hTRT was expressed in all GISTs that showed an activation of telomerase (Fig. 3; Table 2). Compared with the other sarcomas, there was a strong association between hTRT expression and activation of the telomerase in GISTs ($P < 0.0001$). This association could not be observed in leiomyosarcomas ($P = 0.091$) and MPNSTs ($P = 0.231$). In leiomyosarcomas, the hTRT subunit was expressed only in those cases with high levels of telomerase activity (Fig. 4). Eleven leiomyosarcomas with weak telomerase activity did not express hTRT. With some exceptions, the same situation was given in MPNSTs.

**DISCUSSION**

Activation of telomerase is a hallmark of carcinogenesis in different tumors. Besides various carcinomas (32–34), telomerase activity was also found in sarcomas. In a study on telomerase activity in liposarcomas, Schneider-Stock et al. (27) pointed out that the activation of telomerase seems to be characteristic of poorly differentiated liposarcomas. Sakurai et al. (35) were the
first to investigate telomerase activity in GISTs. They found an association between telomerase activity and the grading of these tumors. All GISTs showing an activation of telomerase were large, high-risk tumors with a poor prognosis. This result was corroborated by our own investigation. Except for two malignant gastric stromal tumors, all of the other tumors tended to show high telomerase activity. In two benign GISTs examined in our study, however, there was no telomerase activity. Interestingly, in one case of malignant stromal tumor of the small intestine, the primary tumor showed no activity at first but surprisingly, in one case of malignant stromal tumor of the small intestine, the primary tumor showed no activity at first but displayed a marked activity in its recurrence. Schneider-Stock et al. reported a similar phenomenon in four myxoid liposarcomas, which showed telomerase activity only in their recurrences or metastases. The same study showed that almost all low grade liposarcomas were telomerase negative. The two malignant GISTs without telomerase activity mentioned above had a tumor diameter of 6.5 and 7.5 cm, respectively. Both of them were diploid and showed only a low proliferation rate (<5%). The catalytic subunit hTRT was not expressed in these tumors. One of the tumors had already developed liver metastases. Because we did not retrieve any tumor tissue from the metastases, we can only speculate about an activation of telomerase in these tumors. Sakurai et al. (35) did not find telomerase activity in two of their “high risk” GISTs as well. They consider these facts to be suggestive of telomerase activation as a late event in GIST. Bednarek et al. (36) reported a late activation of telomerase in the tumorigenesis of chemically induced skin carcinomas in mice. Blasco et al. (37), however, found an initial up-regulation of telomerase activity in tumorigenesis.

A further aim of our study was to check the expression of the telomerase subunits hTR and hTRT. Because it has been shown that the telomerase-associated protein TP1, the third subunit of telomerase, does not influence the enzyme activity (38), and that only a posttranscriptional modification of the protein may affect telomerase activity (39), we did not investigate our series of tumors for the expression of TP1.

hTR, the RNA component of telomerase, was always positive in all GISTs, independent of its biological behavior and the clinicopathological data, as well as in all MPNSTs and leiomyosarcomas. There was no correlation between the expression of hTR and telomerase activity. Although several investigators could almost always show an increased expression of hTR in malignant tumors (40–42), there is obviously no correlation of hTR and the level of telomerase activity in each case (43).

A certain correlation exists between telomerase activity and the expression of the catalytic subunit hTRT (44–46). That hTRT is in fact a critical determinant in the activation of telomerase is reflected in the fact that there is a strong association between the expression of hTRT and telomerase activity in all GISTs. Both in leiomyosarcomas and MPNSTs, the relationship between hTRT expression and activation of telomerase was not seen in all cases. The leiomyosarcomas showed an expression of hTRT in those tumors having high telomerase activity. Telomerase activity was found in six MPNSTs not showing expression of hTRT. This discordance was explained by the theory that other factors may substitute for hTRT in conferring full telomerase activity (47). Only one MPNST expressed hTRT without activation of telomerase. In this case, two possibilities were suggested: (a) posttranscriptional modification of the subunits could play a role in the regulation of telomerase activity; (b) different expression levels of each subunit and/or the balance of the expression levels between the subunits might play a critical role in determining enzymatic activity (47).

In conclusion, compared with extragastrointestinal myogenic or neurogenic sarcomas, malignant GISTs express telomerase at a significantly higher level. Benign GISTs show no enzymatic activity. However, a close correlation between telomerase activity and hTRT does exist. That telomerase is reactivated only in a recurrence suggests that the activation of this enzyme is a late event and possibly related to tumor progression.

Although all soft tissue tumor entities investigated in this study resemble each other by conventional histology, there are...
obviously remarkable differences in the telomerase activity and in the expression of the telomerase subunits. In contrast to other soft tissue tumors, a final consensus on grading GISTs has not been achieved, and the biological behavior is often uncertain, leading to problems in the management of these tumors. Therefore, it is necessary to find new markers that are able to shed light on this matter. Telomerase activity and the expression of the telomerase subunit hTRT can give a hint to the tumor behavior. Further investigations are necessary to validate these results as a prognostic factor.

ACKNOWLEDGMENTS

We thank Diana Handke and Gabi Domnick for excellent technical assistance. We are grateful to Bernd Wuesthoff for editing the manuscript.

REFERENCES

Telomerase Activity and Expression of hTRT and hTR in Gastrointestinal Stromal Tumors in Comparison with Extragastrointestinal Sarcomas

Thomas Günther, Regine Schneider-Stock, Carslen Häckel, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/5/1811

This article cites 41 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/5/1811.full.html#ref-list-1

This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/6/5/1811.full.html#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.