Close Correlation between Telomerase Expression and Adenomatous Polyp Progression in Multistep Colorectal Carcinogenesis

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Abstract

To investigate the role of telomerase in the multistep colorectal carcinogenesis, we examined telomerase activity in 31 adenomatous polyps and 22 paired cancer-normal mucosa specimens from non-hereditary nonpolypsis colorectal cancer patients. Telomerase activity was detected in 18% of normal mucosa, 16% of small (<1.0 cm) polyps, 20% of intermediate polyps, 71% of large (>2.0 cm) polyps, and 96% of adenocarcinoma samples (P for trend, <0.0001). High-level enzyme activities were seen in none of the normal mucosa, 5% of small polyps, 20% of intermediate polyps, 43% of large polyps, and 73% of adenocarcinoma samples (P for trend, <0.0001). These data indicate telomerase reactivation occurs with adenomatous polyp progression in multistep colorectal carcinogenesis.

Introduction

Telomeres are the protein-DNA structures at the ends of eukaryotic chromosomes. They allow the cell to distinguish intact from broken chromosomes and to protect chromosomes from degradation, and they are substrates for novel replication mechanisms (1). Telomerase is a ribonucleoprotein polymerase that adds telomeric sequences to chromosome ends (2). It has been proposed that somatic cells are deficient in telomere maintenance and that loss of terminal sequences with each round of DNA replication is the process that records their proliferative history, whereas short telomeres provide the signal for growth arrest at senescence (3). Avoidance of telomeric shortening by expression of telomerase may contribute to the immortal phenotype (4, 5). Carcinogenesis is a multistep process characterized by multiple genetic changes. The development of colorectal neoplasia occurs in a series of genetic steps that correspond to the histological progression from normal colonic epithelium to adenoma to carcinoma. The reported incidence of detection of telomerase activity in colorectal adenoma has varied from 0–100% (6–8). Because the malignant potential increases as the adenoma progresses and increases in size (9–10), it seems crucial to evaluate the association between telomerase activity and polyp size to clarify the role of telomerase reactivation in colorectal carcinogenesis. We found previously (11) that telomerase was expressed in a relatively high proportion (33.5%) of normal mucosal tissues from HNPCC patients, suggesting that genetic defects in HNPCC individuals facilitate the telomerase reactivation in the very early stage of carcinogenesis. We have now expanded our analysis to a larger number of specimens of adenomatous polyps and non-HNPCCs. We report here the first evidence that telomerase expression is closely correlated with polyp progression in multistep colorectal carcinogenesis.

Materials and Methods

Tumors, Tissues, and Cell Culture. A total of 75 tissue samples were used in this study including 22 paired cancer-normal mucosal specimens (obtained from 22 non-HNPCC patients) and 31 adenomatous polyp specimens (from 23 patients, 9 with and 14 without colorectal cancer). Each polyp sample was bisected, and one-half was processed for routine histopathological examination, and the other was stored at −80°C until it was tested for telomerase activity. A normal mucosa specimen was obtained at least 10 cm away from the margin of the tumor. The histological confirmation of each sample was performed using a frozen section stained with H&E. HeLa cells were grown at 37°C, 5% CO2 in DMEM containing 10% fetal bovine serum and antibiotics (100 units/ml penicillin, 100 units/ml streptomycin, and 0.25 μg/ml amphotericin B).

Telomerase Assay. Telomerase assays were performed as described previously (11). In brief, aliquots of tissue extract containing 0.05–5.0 μg were added to 50-μl reaction mixture. Positive telomerase activity in an extract was determined by the presence of a six-nucleotide ladder of TRAP assay products in PAGE that were sensitive to RNase pretreatment. For each run of the assay, the HeLa extracts with three protein concentrations ranging from 0.005–0.5 μg/50 μl reaction mixture were assayed in parallel to serve as positive control for comparison. Data were collected blindly and decoded only after the assays were completed. The level of telomerase activity in a tissue extract was determined using the data obtained from 0.5 μg of protein extract. Telomerase activity was scored as described previously (11). Moderate to strong activity was designated as high telomerase level. Weak or undetectable activity was designated as low level.

Statistics. Fisher’s exact test was used to compare categorical data between two groups. The Mantel-Haenszel test was used to test for linear association. All of the Ps resulted from two-sided tests. Multivariate analysis with a multiple logistic regression model was used to identify independent variables associated with telomerase expression. This analysis was performed using SPSS for Windows Software (Release 7.0, SPSS Inc., Chicago, IL).

Results

We found that both the intensity and frequency of detectable telomerase activity increased with polyp size. Five (72.4%) of 7 polyps with a size of >2 cm had detectable telomerase activity, whereas only 3 (15.8%) of 19 polyps with a size <1 cm had such an activity (Table 1; P = 0.030, Fisher’s exact test; P for trend = 0.013). Fig. 1 shows the gross colorectal specimen and the location of the six polyps from which the respective samples were obtained for telomerase analyses in a case of familial adenomatosis polyposis. The size of the six polyps ranged from 0.3–6.0 cm. The intensity and frequency of detectable telomerase activity increased with increasing polyp size (Fig. 2). Telomerase activity in relation to pathological variables for the 31 polyps is listed in the Table. Seven (53.8%) of 13 polyps associated with a synchronous cancer had detectable activity compared with 2 (11.1%) of 18 polyps without a synchronous cancer (P = 0.017). Multivariate logistic regression analysis including two covariates (associated with cancer and polyp size) revealed that only the polyp size (tumor size <1.0 versus ≥1.0 cm; odds ratio, 1.0 versus...
entire mucosa of the colon. The size of tumors 1-5 was 0.3, 0.6, 0.8, 1.8, and 4.1 cm, respectively. AN, anus; AP, appendix.

The sigmoid colon. The rectum was relatively spared from polyposis, which carpeted the rectum. An ulcerative rectal cancer (tumor 6, size = 6.0 cm) was benign and located at the sigmoid colon. The rectum was relatively spared from polyposis, which carpeted the entire mucosa of the colon. The size of tumors 1-5 was 0.3, 0.6, 0.8, 1.8, and 4.1 cm, respectively. AN, anus; AP, appendix.

Of 22 paired cancer-normal mucosa specimens, 21 (95.5%) cancer specimens had detectable telomerase activity (including 7 with strong activity, 9 with moderate activity, and 5 with weak activity), whereas 3 (18.2%) normal mucosa specimens had a detectable telomerase activity (all with weak activity). Fig. 3 shows that the prevalence of high-level telomerase activity increased as polyps enlarged and progressed from normal colonic epithelium to small adenoma to large adenoma and to cancer (P for trend, <0.0001).

To compensate for the lower sensitivity of the non-radioisotope assay used in this study as compared with the radioisotope assay, the results of tissue samples that did not display telomerase activity were confirmed by repeating the TRAP assay using 0.05 µg and 5 µg extract protein in the reaction mixtures. To exclude the possibility that an undetectable telomerase activity could be caused by the existence of assay inhibitors, we repeated the TRAP assay using 0.05 µg and 5 µg extract protein in the reaction mixtures and mixed the telomerase-negative extracts with the HeLa cell extract (see "Materials and Methods"). Because contamination of lymphocyte infiltration in the tissues may account for a false-positive telomerase activity, a histological review of all of the polyps with a size >1.0 cm was performed. These polyps were histologically similar in degree of lymphocyte infiltration.

![Image](image_url)

**Table 1 Relation between telomerase expression and clinicopathological variables in 31 polyps**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>n</th>
<th>Undetectable n (%)</th>
<th>Detectable n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>&lt;50</td>
<td>10</td>
<td>6 (60.0)</td>
<td>4 (40.0)</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>≥50</td>
<td>21</td>
<td>16 (76.2)</td>
<td>5 (23.8)</td>
<td>0.030†</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>16</td>
<td>10 (62.5)</td>
<td>6 (37.5)</td>
<td>0.660</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>15</td>
<td>12 (80.0)</td>
<td>3 (20.0)</td>
<td>0.433</td>
</tr>
<tr>
<td>Synchronous cancer</td>
<td>Absent</td>
<td>18</td>
<td>16 (88.9)</td>
<td>2 (11.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>13</td>
<td>6 (46.2)</td>
<td>7 (53.8)</td>
<td>0.017</td>
</tr>
<tr>
<td>Polyp site</td>
<td>Colon</td>
<td>23</td>
<td>17 (73.9)</td>
<td>6 (26.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>8</td>
<td>5 (62.2)</td>
<td>3 (37.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>Polyp size (cm)</td>
<td>&lt;1</td>
<td>19</td>
<td>16 (84.2)</td>
<td>3 (15.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>5</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>7</td>
<td>2 (28.6)</td>
<td>5 (71.4)</td>
<td>0.030†</td>
</tr>
</tbody>
</table>

*Test for trend, P = 0.013.

**Discussion**

Fearon and Vogelstein (12) were first to propose a genetic model for multistep colorectal tumorigenesis. The identification of an intermediate in the progression pathway, the adenomatous polyp, is one of the reasons for the detailed knowledge about the multistep nature of this system. Most investigators now favor the hypothesis that telomerase reactivation is unlikely to have occurred in most adenomas and that the telomerase activity detected in all of the adenomas in previous reports was likely derived from normal crypt cells (13). Polyp size plays an important role in colorectal adenoma-carcinoma transformation (9–10). Muto et al. (9) reported that adenomatous polyps under 1.0 cm have a 1% incidence of malignant change; those from 1.0-2.0 cm have a 10% incidence, and those over 2.0 cm have a 35% incidence.

In this study, weak enzyme activity was expressed in a subset (18%) of the 22 corresponding normal mucosa samples from colorectal cancer patients. Telomerase activity was present in 96% of cancer lesion samples. Most (73%) of the cancer samples expressed high-level enzyme activities. In adenomatous polyps, a greater incidence of telomerase activity was noted in large polyps. Compared with smaller polyps, the odds of detecting telomerase activity in a polyp of size ≥1.0 cm was 14.2. There was a linear relationship between polyp size and telomerase activity. Most samples of telomerase-positive small
POLYPS EXPRESSED WEAK ENZYME ACTIVITIES. HOWEVER, A SUBSTANTIAL PORTION (43%) OF LARGE POLYPS (>2 CM) EXPRESSED HIGH-LEVEL ENZYME ACTIVITIES. A HIGHLY SIGNIFICANT LINEAR CORRELATION WAS FOUND BETWEEN POLYP PROGRESSION (FROM NORMAL COLONIC EPITHELIUM TO SMALL ADENOMA TO LARGE ADENOMA AND TO CANCER) AND HIGH-LEVEL TELOMERASE ACTIVITY. THE LINEAR RELATIONSHIP DID NOT SEEM TO BE THE RESULT OF NORMAL CRYPT CELLS OR LYMPHOCYTES IN THE POLYP SAMPLES BECAUSE THE TELOMERASE-POSITIVE AND -NEGATIVE POLYPS WERE HISTOLOGICALLY SIMILAR IN TERMS OF LYMPHOCYTE INFILTRATION. MOREOVER, LARGER POLYPS WERE LESS LIKELY TO CONTAIN NORMAL CRYPT CELLS. THE REACTIVATION OF TELOMERASE IS NECESSARY FOR SOMATIC HUMAN CELLS TO MAINTAIN THE LENGTH OF THEIR TELOMERES (4-5). YET, THE DETECTION OF TELOMERASE ACTIVITY ONLY INDICATES THE INCREASED POTENTIAL FOR CELLULAR IMMORTALITY AND IS NOT ALWAYS SYNONYMOUS WITH THE ACQUISITION OF CANCER (14). ONE STUDY (15) ALSO SUGGESTED THAT TELOMERASE ACTIVITY IS A BIOMARKER OF CELL PROLIFERATION RATHER THAN OF MALIGNANT TRANSFORMATION. RECENT EVIDENCE SUGGESTS THAT THE REACTIVATION OF TELOMERASE HAS OCCURRED IN PREMALIGNANT LESIONS DURING CARCINOGENESIS IN THE CANCER OF OTHER ORGANS, SUCH AS LUNG CARCINOMA (16), OVARIAN CANCER (17), ORAL CARCINOMA (18), AND CERVICAL CANCER (19).

THE RESULTS OF THIS STUDY SUGGEST THAT POLYP PROGRESSION (FROM NORMAL EPITHELIUM TO ADENOMA TO CANCER) IS ASSOCIATED WITH INCREASED FREQUENCY AND INTENSITY OF TELOMERASE ACTIVITY. TELOMERASE REACTIVATION OCCURS AT AN EARLY STAGE IN THE PATHOGENESIS OF COLORECTAL CANCERS. THE ABRUPTION OF CELLULAR SENESCENCE THROUGH TELOMERASE REACTIVATION MAY BE NECESSARY FOR THE CONTINUED GROWTH OF POLYPS AND ANY TRANSITION TO MALIGNANCY.

REFERENCES

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