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Short Communication

Telomerase Activity Is Commonly Detected in Hereditary Nonpolyposis Colorectal Cancers

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Telomerase activity can be detected in most human cancers. These findings are consistent with the telomere hypothesis, which predicts telomerase expression after a number of mitotic divisions to prevent the progressive and catastrophic loss of telomeres. However, telomerase is not detected in a minority of colorectal cancers suggesting either alternative mechanisms of immortalization or that their telomeres have not yet shortened sufficiently to require telomerase activity. Colorectal cancers arising in patients with hereditary nonpolyposis colorectal cancer (HNPCC) were examined for telomerase activity because compared to sporadic tumors, HNPCC tumors are less likely to pass a telomere threshold as they occur in younger patients and exhibit "accelerated" progression, perhaps because of their characteristic mutator phenotypes and losses of mismatch repair. Primary colorectal cancers, 13 in HNPCC patients, and 37 sporadic tumors (17 with mutator phenotypes) were examined for telomerase activity by the TRAP (telomeric repeat amplification protocol) assay. The majority of colorectal cancers contained detectable telomerase activity regardless of underlying phenotype (77% of HNPCC; 81% of sporadic tumors, 88% with mutator phenotypes and 75% without mutator phenotypes). Therefore, telomerase expression appears to be commonly acquired in the progression of both mutator phenotype and sporadic colorectal cancers. (Am J Pathol 1996, 148:1075–1079)

Chromosomes normally shorten with mitosis because of the inherent inability of DNA polymerases to replicate telomeres.[1,2] Decreases in telomere sizes are observed with aging and replication of primary cell cultures.[3,4] Progressive reduction of telomere lengths to critically short sizes has been correlated with the cessation of cell division and the onset of senescence.[5] Immortalization of primary and tumor cell lines is associated with stable telomere sizes despite mitotic activity, and the expression of telomerase.[5–8] Telomerase is a ribonucleoprotein that extends telomeres by the addition of six base pair direct repeats.[9]

Telomerase activity is absent from most human tissues but expressed in the majority of cancers.[6] Carcinogenesis is a multistep process involving the accumulation of an unknown number of mutations and an unknown number of cell divisions between initiation and clinical presentation.[10] The colorectal adenoma-carcinoma sequence is one of the best characterized models for multistep tumorigenesis. Progression may be extremely slow as the ratio of adenomas to cancers is high.[11] Telomere shortening is observed in colorectal adenomas and carcinomas.[12] Recent studies have demonstrated that although most adenomas lack telomerase activity, the majority of colorectal cancers express this enzyme.[6,13]

These findings in colorectal cancer are consistent with the telomere hypothesis.[5] The number of cell divisions necessary to progress to cancer may result in the critical shortening of telomeres. Further progression past this point would require the expression of telomerase to prevent catastrophic telomere loss. However, a minority of colorectal cancers lack de-

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tectable telomerase activity[6,13] and other alternatives remain.[8] Hereditary nonpolyposis colorectal cancers (HNPCC) comprise approximately 5% of all colorectal cancers (similar to the fraction of telomerase negative colorectal cancers) and arise at an earlier age compared with sporadic tumors.[14] Although most HNPCC tumors arise from adenomas, the progression to carcinoma appears to be accelerated, as the ratio of adenomas to carcinomas is low and a high proportion of HNPCC adenomas exhibit dysplastic changes.[14–18] Furthermore, the underlying genetic basis for HNPCC are germline mutations in mismatch DNA repair genes.[19–23] The loss of mismatch repair in HNPCC tumors results in a mutator phenotype manifested by the accumulation of thousands of somatic mutations in simple repeat sequences or microsatellites (MS).[19,24] The increased mutation rates observed in repair deficient cell lines[24–26] provide a mechanism for the acceleration of tumor progression, as fewer cell divisions may be necessary to accumulate a critical number of mutations.[27] Because these factors may favor malignant transformation before passage past a telomere threshold, telomerase activity was compared between HNPCC tumors and sporadic colorectal cancers with or without mutator phenotypes.

Materials and Methods

Specimens

Primary colorectal cancers were frozen and stored at −70°C. Of the 50 tumors, 13 were from HNPCC families with verified germline mutations and/or MS instability in their tumors (unpublished data).[19,28,29] Patients with the 37 sporadic tumors lacked histories consistent with HNPCC. The sporadic tumors had been previously examined for the presence mutator phenotypes manifested by somatic alterations of MS (unpublished data).[19,28,29]

Telomerase Assay

Single 5–10 micron thin cryostat frozen tissue sections approximately 1 cm^2 in size were obtained from the colorectal cancers. Histological examination verified that tumor cells were at least 50% of all cells. The single tissue sections were extracted and analyzed for telomerase activity by the telomerase repeat amplification protocol (TRAP) assay.[6] Approximately 5–10% of each protein extract was subjected to the TRAP assay. PCR products were labeled with ^{33}PdCTP present in the reaction mix. A total of 40 PCR cycles were utilized to enhance the sensitivity of the assay for the small amounts of tumor tissue present in the single tissue slices. This modified TRAP assay failed to detect telomerase from primary human fibroblasts or normal colon (n = 6) but could detect as few as 10 telomerase positive tumor cells mixed with 100,000 telomerase negative primary fetal foreskin fibroblast cells (data not shown). For all telomerase positive specimens, RNAse digestion confirmed the expected loss of telomerase activity. The absence of inhibitors was tested by adding the equivalent of 50 telomerase-positive tumor cells to all negative specimens to demonstrate the expected amplification.

Results

A series of 50 primary colorectal cancers was examined in parallel for telomerase activity using the PCR based TRAP assay.[6] Two groups of tumors were examined—13 from HNPCC patients and 37 from patients without HNPCC. The sporadic tumors were further divided into approximately equal numbers with (n = 17) and without (n = 20) mutator phenotypes manifested by ubiquitous somatic mutations in the majority of tested microsatellite loci.[30] The average age at clinical presentation was 48.5 years for the HNPCC patients and 70.0 years for the patients with sporadic cancers. Overall, the majority (80.0%) of the colorectal cancers exhibited detectable levels of telomerase (Figure 1). The lower frequency of telomerase detection compared with other studies of colorectal cancer[6,13] may reflect possible tumor heterogeneity with respect to telomerase expression,[31] as only a small portion of each tumor was examined. There were no significant differences in the frequencies between the groups with 76.9% of HNPCC and 81.1% of sporadic (88.2% with mutator phenotypes) tumors having detectable telomerase (Table 1). The positive signal intensities of the characteristic six base pair repeat ladders did not differ between the positive HNPCC and sporadic tumor specimens. There was also no apparent correlation between stage and telomerase detection (Table 2). One adenoma from an HNPCC

Table 1. *Telomerase Expression*

Type	Number	Telomerase-positive		Age	Sex M	Sex F
HNPCC	13	10	76.9%	48.5	9	4
Sporadic	37	30	81.1%	70.0	19	19
Mutator Phenotype+	17	15	88.2%	67.7	6	11
Mutator Phenotype−	20	15	75.0%	72.0	13	7

Table 2. *Telomerase Expression: Stage and Age*

Type	Duke's stage A	B	C	D	Age
HNPCC					48.5
Telomerase+	5	3	2	0	46.7
Telomerase−	1	2	0	0	54.7
Sporadic					70.0
Telomerase+	1	21	8	0	70.1
Telomerase−	0	3	2	2	69.7

patient was available for examination and was negative for telomerase.

Discussion

The detection of telomerase activity in the majority of human tumors suggests that the number of cell divisions between tumor initiation and clinical presentation is usually sufficient to critically shorten telomeres past a threshold such that cellular death or senescence will occur unless telomerase is expressed. An alternative explanation is that the process of tumorigenesis or immortalization activates telomerase secondarily.[8] The HNPCC tumors examined in this study are less likely to pass a mitotic telomere threshold compared with sporadic colorectal cancers for several reasons. First, the HNPCC tumors presented at an earlier age and therefore their base line telomere lengths at initiation are likely to be longer than the sporadic tumors. Second, HNPCC tumorigenesis appears to be greatly accelerated compared with sporadic tumors,[14,15,17] suggesting that the number of divisions between initiation and clinical presentation may be less than sporadic tumors. Finally, telomere shortening can destabilize chromosomes, which may contribute to tumor progression and chromosomal abnormalities[5]. In contrast, mutator phenotype tumors tend to be diploid or near diploid, and genomic instability occurs very early in tumor progression through the loss of DNA mismatch repair.[19–21,25,28,30,32] Mutator phenotype colorectal cancers also exhibit significantly better clinical outcomes compared with similar stage nonmutator tumors.[28,32,33] Lack of telomerase expression may account for the better prognosis, as recently observed in neuroblastomas.[34]

The detection of telomerase activity in the majority of HNPCC and sporadic mutator phenotype tumors is consistent with the general correlation between telomerase expression and most cancers. Therefore, colorectal cancers with mutator phenotypes do not fundamentally differ from nonmutator cancers with respect to telomerase expression. Unfortunately, this finding is not

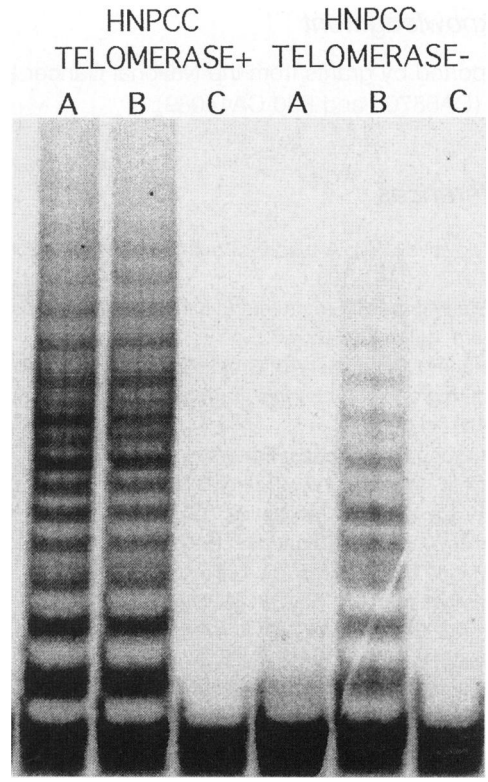

Figure 1. *Autoradiograph of TRAP PCR products illustrating telomerase-positive and telomerase-negative HNPCC specimens.* **A Lanes** *are the results from the specimens.* **B Lanes** *are the results of the specimens and added tissue culture cells, demonstrating the lack of inhibitors in the telomerase negative specimen.* **C Lanes** *are the results after incubation of the specimens with RNAse, demonstrating the expected loss of telomerase activity.*

informative on the relationship between telomerase expression and telomere shortening. This question could be better answered by direct measurements of telomere lengths at the onset of telomerase expression. However, no direct relationship exists between telomere lengths and telomerase expression in tumors, perhaps because telomerase expression can lead to telomere repair.[6,7,31] Although clinical studies have previously noted that HNPCC adenomas have greater proclivity for malignant degeneration compared with sporadic adenomas,[14,16,18] a large and currently unknown number of cell divisions, sufficient to require telomerase expression, may still intervene between initiation and clinical presentation. Alternatively, telomeres may erode faster in the setting of deficient DNA mismatch repair and thereby reach a threshold after fewer divisions.

In summary, most HNPCC and sporadic mutator colorectal cancers express telomerase. Although multiple mechanisms are possible, the findings demonstrate that colorectal cancers with high mutation rates still contend with the chromosome end replication problem.

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