Somatic mutations in telomerase promoter counterbalance germline loss-of-function mutations

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Germline coding mutations in different telomere-related genes have been linked to autosomal-dominant familial pulmonary fibrosis. Individuals with these inherited mutations demonstrate incomplete penetrance of clinical phenotypes affecting the lung, blood, liver, skin, and other organs. Here, we describe the somatic acquisition of promoter mutations in telomerase reverse transcriptase (TERT) in blood leukocytes of approximately 5% of individuals with inherited loss-of-function coding mutations in TERT or poly(A)-specific ribonuclease (PARN), another gene linked to telomerase function. While these promoter mutations were initially identified as oncogenic drivers of cancer, individuals expressing the mutations have no history of cancer. Neither promoter mutation was found in population-based cohorts of similar or advanced age. The TERT promoter mutations were found more frequently in cis with the WT allele than the TERT coding sequence mutation. EBV-transformed lymphoblastoid B cell lines (LCLs) derived from subjects with TERT promoter mutations showed increased telomerase expression and activity compared with cell lines from family members with identical coding mutations. TERT promoter mutations resulted in an increased proliferation of LCLs and demonstrated positive selection over time. The persistence and recurrence of noncoding gain-of-function mutations in these cases suggests that telomerase activation is not only safely tolerated but also advantageous for clonal expansion.

Introduction
Telomerase is a reverse transcriptase that extends the length of repetitive DNA sequences at the ends of chromosomes (1). In contrast to normal tissue, in which telomerase activity is absent, most human tumors (85%–90%) constitutively express telomerase (2). Activating germline mutations in the telomerase reverse transcriptase (TERT) promoter are some of the most common mutations found in human cancer, occurring frequently in bladder cancer, melanoma, and glioblastoma (3). The most common TERT promoter mutations are located at positions −124 and −146 upstream of the TERT coding start site. These mutations create a transcription binding site for GABP, an ETS transcription factor (4), and lead to increased TERT expression, telomerase activity, telomere length, and tumor formation (5, 6).

Coding mutations in TERT as well as other genes in the telomere pathway (telomerase RNA component [TERC], poly(A)-specific ribonuclease [PARN], regulator of telomere elongation helicase 1 [RTEL1], and nuclear assembly factor 1 ribonucleoprotein [NAF1]) have been found in patients with familial pulmonary fibrosis and idiopathic pulmonary fibrosis (IPF) (7–10). Pathogenic rare variants in TERT are found in approximately 15% of familial cohorts and approximately 3% of sporadic IPF patients; mutations are more commonly found in TERT than in any other gene. Additionally, all pathogenic rare variants lead to reduced protein function, decreased telomerase enzymatic activity, and shortened telomere lengths.

While adult-onset pulmonary fibrosis is common, the clinical spectrum of extra-pulmonary manifestations is broad and overlaps dyskeratosis congenita (DC). We hypothesized that the acquisition of somatic gain-of-function promoter mutations might counteract the effect of germline loss-of-function mutations.

Results and Discussion
The TERT promoter was sequenced from DNA isolated from blood leukocytes of 199 subjects, each with a previously identified heterozygous germline mutation in one of 4 telomere-related genes (TERT, TERC, PARN, and RTEL1, Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI91161DS1) associated with pulmonary fibrosis. The individuals, 50% of whom were male, ranged in age from 15 to 84 years (mean 51 ± 16 years). Table 1 lists the 10 subjects (5%) in whom a TERT promoter mutation was found. All but 1 of these 10 subjects was non-Hispanic white (NHW), all but 1 was male, and the mean age was 54 ± 7 years. Nine of one hundred thirty-seven (6.5%) with a germline mutation in TERT and one of thirty-two (3.1%) with a germline mutation in PARN had one of two different TERT promoter mutations (−124C>T and −146C>T) in blood leukocytes. There was no history of cancer in these 10 individuals. Most had pulmonary fibrosis, one was asymptomatic, and one was lost to follow up. We found no difference in leukocyte telomere lengths in subjects with TERT promoter and coding mutations compared with family members with an identical coding mutation as measured by quantitative PCR (qPCR) or flow-FISH (data not shown). The total white blood cell counts of the TERT promoter mutation carriers were within the normal range, although some of the lym-
To determine the frequency of the TERT promoter mutations in blood from control subjects, we screened the population-based Dallas Heart Study cohort (Supplemental Figure 1). None of the 2,611 subjects (mean age of 50 ± 11 years, including 895 NHW and 1,102 males) was found to carry either of these 2 TERT promoter mutations. Thus, 9 of the 179 NHW telomere-related mutation carriers and none of the 895 NHW control subjects were found to have a TERT promoter mutation (P = 8.36 × 10⁻⁸ by Fisher’s exact test). In addition, none of the 132 individuals from advanced-age control populations, ranging in age between 60 and 90 years, were found to have mutations in the TERT promoter.

phocyte subsets were variably low, consistent with the spectrum of immunological abnormalities found in these individuals (Supplemental Tables 2 and 3). Several had evidence of macrocytosis, which is commonly seen in pulmonary fibrosis patients with germ-line telomere-related gene mutations (11).

The promoter mutations in TERT were not found in DNA isolated from skin fibroblasts or buccal epithelium (Table 1 and Figure 1B). As would be expected with somatic mutations, none of the progeny of the individuals listed in Table 1 were found to have the promoter mutation (data not shown).

Table 1. Blood leukocyte TERT promoter somatic mutations found in subjects with various heterozygous germline mutations in telomere-related genes

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>TERT promoter somatic mutation</th>
<th>Blood</th>
<th>Skin</th>
<th>Germline coding sequence mutation</th>
<th>Clinical phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT822</td>
<td>58</td>
<td>M</td>
<td>–124C&gt;T/–146C&gt;T</td>
<td>Present</td>
<td>ND</td>
<td>TERT c.430G&gt;A (Val144Met, exon 2)</td>
<td>Died, pulmonary fibrosis</td>
</tr>
<tr>
<td>UT2932</td>
<td>61</td>
<td>M</td>
<td>–146C&gt;T</td>
<td>Present</td>
<td>Absent</td>
<td>TERT c.1710G&gt;T (Lys570Asn, exon 3)</td>
<td>Pulmonary fibrosis, s/p lung transplant</td>
</tr>
<tr>
<td>UT2031</td>
<td>48</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>Absent</td>
<td>TERT c.1892G&gt;A (Arg631Gln, exon 4)</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>UT3224</td>
<td>47</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>Absent</td>
<td>TERT c.1895C&gt;T (Pro632Leu, exon 4)</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>UT387</td>
<td>52</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>Absent</td>
<td>TERT c.2240delT (Val747Valfs, exon 6)</td>
<td>Died, pulmonary fibrosis</td>
</tr>
<tr>
<td>UT248</td>
<td>47</td>
<td>F</td>
<td>–146C&gt;T/–124C&gt;T</td>
<td>Present</td>
<td>Absent</td>
<td>TERT c.2594G&gt;A (Arg865His, exon 9)</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>UT2210</td>
<td>51</td>
<td>M</td>
<td>–146C&gt;T</td>
<td>Present</td>
<td>ND</td>
<td>TERT c.592-2A&gt;G (splice acceptor site, exon 9)</td>
<td>LOST to follow-up</td>
</tr>
<tr>
<td>UT2482</td>
<td>50</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>ND</td>
<td>TERT c.2627T&gt;C (Phe883Ile, exon 10)</td>
<td>Died, pulmonary fibrosis</td>
</tr>
<tr>
<td>UT3410</td>
<td>63</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>ND</td>
<td>TERT c.2991delC (Val997Valfs, exon 13)</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>UT986</td>
<td>64</td>
<td>M</td>
<td>–124C&gt;T</td>
<td>Present</td>
<td>ND</td>
<td>PARN c.529C&gt;T (Gln177Ter)</td>
<td>Died, sepsis</td>
</tr>
</tbody>
</table>

F, female; M, male; ND, not determined; s/p, status post.
and Supplemental Table 4). Thus, there is a bias toward selection of cells in which the activating promoter mutation is driving the expression of the WT, but not the mutant, telomerase allele.

We used cell lines to test whether the promoter mutation found in cis with the WT allele is associated with increased telomerase activity. The EBV-transformed lymphoblastoid B cell line (LCL) derived from the individual carrying the C>T mutation at position –146 in the TERT promoter (subject UT248) had higher telomerase activity and higher human TERT gene expression (Figure 3, A and B) than did family members with the same TERT coding sequence mutation. The mutant –146C>T peak was less prominent in slowly proliferating cells than in rapidly proliferating cells after approximately 1 week (Figure 3C). The relative abundance of the –146 C>T mutation was much higher after 46 weeks in culture, indicating a preferential selection for this mutant allele (Figure 3D). LCLs isolated from carriers with only the TERT coding sequence mutation did not acquire the TERT promoter mutations over time (data not shown). We also noted increased telomerase activity and human TERT expression for subject UT3410, who carries the –124C>T somatic promoter mutation, when compared with family member controls (Supplemental Figure 4, A and B). We observed similar increases in the promoter mutation peak height, especially for cell lines cultured over long periods of time (Supplemental Figure 4, C and D). As LCLs often demonstrate genomic instability, we suspect that aneuploidy and cellular heterogeneity lead to enrichment of the mutant allele.

These data suggest that spontaneous acquisition of TERT promoter mutations are selected for in the somatic blood cells of individuals with short telomere lengths and heterozygous germ-
rather than passenger events (15, 16). These mutations are associated with resistance to standard therapies, metastasis, recurrence, and poorer survival (17). As is seen in cancer studies, the frequency of the –124C>T mutation (80%) is usually overrepresented compared with the frequency of the –146C>T promoter mutation (20%). To our knowledge, these somatic TERT promoter mutations have not been previously described in human cohorts without cancer. None of the individuals with a TERT promoter mutation in this study had a personal history of cancer, but they will need to be carefully monitored for neoplastic events, as cancers are found at a higher rate in patients with DC than in the general population (18). It is possible that these somatic mutations may be better tolerated in certain cell types, such as granulocytes or B cells, in individuals with germline telomere–related mutations.

Hematopoietic stem/progenitor cells are dividing continuously and accumulating rare random mutations, many of which are age related and associated with clonal expansion (19). In subjects with germline mutations in the telomerase complex, somatic TERT promoter mutations are likely selected by the advantages they provide for self-renewal and proliferation. As granulocytes
and monocytes are short-lived cells, the promoter mutations have probably been acquired by progenitors that have undergone clonal expansion. During infection or vaccination, B cells with the highest affinity for antigen undergo clonal expansion and outp浓缩ate the lower-affinity cells. Future studies would be needed to determine whether the TERT promoter mutations are linked to clonal V (DJ) recombination. This study is congruent with the previously described skewed X-inactivation, somatic reversion, and clonal hematopoiesis found in patients with DC (20, 21). However, in contrast to the revertant mosaicism that leads to spontaneous correction or deletion of a pathogenic allele, here we made multiple independent observations of an activating promoter mutation on the contralateral (WT) allele. In 2 individuals (UT248 and UT822), the presence of 2 different promoter mutations linked to the WT allele underscores the selective force for these compensatory mutations to counteract progressive telomere shortening.

Although rare, these patients provide evidence of noncoding gain-of-function mutations that counterbalance germline loss-of-function mutations. The net effect of these opposing genetic forces likely leads to normalized telomerase activity. Evidence from a recent study demonstrating telomere elongation in response to danazol suggests pharmacologic activation of the telomerase promoter (22). Male sex hormones are known to upregulate telomerase and have been used to treat bone marrow failure, even for patients with DC (23). Additional investigation is warranted to determine the efficacy of telomerase activators in patients with short telomere syndromes, including those with pulmonary fibrosis. The persistence of the somatic mutation for at least 10 years in 1 patient provides genetic evidence that increased telomerase activation may be clinically safe in the setting of germline TERT coding mutations.

Methods

The Methods are detailed in the Supplemental Methods.

Statistics. All data are presented as the mean ± SD. Two-tailed Student’s t tests and Fisher’s exact tests were used to generate P values.

Study approval. The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

Author contributions

RCW, NSCVO, and CKG designed experiments; LM performed sequencing, limiting dilution, TRAP, qPCR, and proliferation experiments. YY performed the limiting dilution assay. CB sequenced the centenarian participants. CAN measured telomere lengths. AY performed sequencing and allelic discrimination. CKG wrote the manuscript. All authors interpreted the data and contributed to the final version of the manuscript.

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BRIEF REPORT

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