TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal


Malignant cells, like all actively growing cells, must maintain their telomeres, but genetic mechanisms responsible for telomere maintenance in tumors have only recently been discovered. In particular, mutations of the telomere binding proteins alpha thalassemia/mental retardation syndrome X-linked (ATRX) or death-domain associated protein (DAXX) have been shown to underlie a telomere maintenance mechanism not involving telomerase (alternative lengthening of telomeres), and point mutations in the promoter of the telomerase reverse transcriptase (TERT) gene increase telomerase expression and have been shown to occur in melanomas and a small number of other tumors. To further define the tumor types in which this latter mechanism plays a role, we surveyed 1,230 tumors of 60 different types. We found that tumors could be divided into types with low (<15%) and high (≥15%) frequencies of TERT promoter mutations. The nine TERT-high tumor types almost always originated in tissues with relatively low rates of self-renewal, including melanomas, liposarcomas, hepatocellular carcinomas, urothelial carcinomas, squamous cell carcinomas of the tongue, medulloblastomas, and subtypes of gliomas (including 83% of primary glioblastoma, the most common brain tumor type). TERT and ATRX mutations were mutually exclusive, suggesting that these two genetic mechanisms confer equivalent selective growth advantages. In addition to their implications for understanding the relationship between telomeres and tumorigenesis, TERT mutations provide a biomarker that may be useful for the early detection of urinary tract and liver tumors and aid in the classification and prognostication of brain tumors.

Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes that are required for chromosomal integrity. Several hundred nucleotides of telomere repeats cap each chromosomal end, and in the absence of telomerase activity, telomeres shorten with each cell division (1). Eventually, uncapped telomeres trigger cell death or senescence. Cancer cells seem to divide ad infinitum and therefore, require some telomere maintenance mechanism to avoid this fate. Because telomerase activity is generally higher in cancer cells than normal cells, it was originally believed that telomerase was somehow activated in cancer cells (2–6). However, it was subsequently realized that telomerase was only active in terminally differentiated cells and that normal stem cells in self-renewing tissues retained telomerase activity (1, 7–9). Because normal stem cells must replicate throughout the long lifetimes of mammals (which can be more than a century in humans), it is clear that such cells must also retain telomerase activity. Because normal stem cells are thought to be the progenitors of cancers, there would be no need to specifically activate telomerase in cancer cells; the enzyme was already active in the precursors, just as were the hundreds of other enzymes and proteins normally required for cell proliferation.

This view was challenged by the discovery of another mechanism for maintaining telomere length (i.e., alternative lengthening of telomeres (ALT)) (10–12). ALT occurs in the absence of telomerase activity and seems to be dependent on homologous recombination. It occurs in a particularly high fraction of certain tumor types, such as sarcomas, pancreatic neuroendocrine tumors, and brain tumors, but rarely in most common tumor types, such as those tumor types of the colon, breast, lung, prostate, or pancreas (13). Why would cancer cells need ALT if telomerase activity was already constitutively active in their precursors? This question was highlighted by the discovery that many ALT cancers harbor mutations in alpha thalassemia/mental retardation syndrome X-linked (ATRX) or death-domain associated protein (DAXX), genes encoding proteins that interact with each other at telomeres (10, 11). Presumably, the absence of functional ATRX/DAXX complexes permits the homologous recombination resulting in ALT. At minimum, these data were compatible with the ideas that there could be a selective advantage for genetic alterations that results in telomere maintenance and that telomerase is not indefinitely activated in all normal stem cell precursors of cancers.

Another challenge to the idea that genetic alterations were not required for telomerase activation in cancer was raised by the finding that mutations of the telomerase reverse transcriptase (TERT) promoter occurred in ~70% of melanomas and in a
small number of tumor cell lines derived from various tissue types (14, 15). Importantly, only 5 of 110 cell lines derived from lung, stomach, ovary, uterus, or prostate cancers harbored TERT promoter mutations, whereas 19 mutations were found among 37 cell lines derived from various other tumor types. This situation is analogous to the situation for ALT, which is infrequently observed in common epithelial cancers but is observed more regularly in tumors derived from nonepithelial cells, particularly sarcomas and brain tumors (13).

These findings prompted us to formulate a hypothesis about the mechanisms responsible for telomerase activity in cancers. We suggest that there are two ways to maintain telomere lengths as cells divide: (i) through epigenetic regulation of telomerase activity, which occurs in stem cells of tissues that are rapidly renewing, and (ii) through somatic mutations that maintain telomere lengths, such as mutations in the TERT promoter or mutations in DAXX or ATRX. Those cancers that originate in tissues that are constantly self-renewing, such as cancers of the epithelia of the gastrointestinal tract and skin or bone marrow, would be unlikely to harbor telomere-maintaining mutations, because telomerase is already epigenetically activated in their precursor cells. In contrast, tumors arising from cells that are not constantly self-renewing, such as neurons, glial cells, fibroblasts, hepatocytes, islet cells, and pancreatic ductal epithelial cells, give rise to tumors that would be unlikely to harbor such mutations. A corollary of this hypothesis is that tumor types exhibiting high frequencies of ALT would also exhibit high frequencies of TERT mutations, and these mutations would be distributed in a mutually exclusive fashion. To test these hypotheses as well as answer other questions related to the role of TERT promoter mutations in various cancer types, we determined the prevalence of TERT promoter mutations in a large number of tumors.

**Results**

We attempted to evaluate at least 20 individual specimens of common tumor types and fewer specimens of rare tumor types, depending on availability of specimens in our laboratories. In those tumor types in which our pilot studies showed a significant number of mutations, additional tumors were evaluated. Malignomas and tumors of the lung, stomach, and esophagus were excluded, because they had already been adequately evaluated in the seminal papers cited (14, 15). When primary tumors rather than cell lines were used, we ensured that the fraction of neoplastic cells was >50% through histopathologic examination of frozen sections of the tissue blocks used for DNA purification. In most cases in which the neoplastic content of the specimen was <50%, we microdissected the lesions to enrich the neoplastic content to >50%. Primers were designed to amplify the region containing the two TERT mutations that were previously described—C228T and C250T—corresponding to the positions 124 and 146 bp, respectively, upstream of the TERT ATG start site (14, 15). The PCR fragments were then purified and analyzed by conventional Sanger sequencing.

In all, we evaluated TERT promoter mutations in 1,230 tumor specimens and identified 231 mutations (77.5%) (Table 1), C228T and C250T mutations accounted for 77.5% and 20.8% of the alterations, respectively (Dataset S1). Additionally, we detected four mutations that had not been observed previously: three C228A mutations and one C229A mutation (Dataset S1). All four of these mutations as well as a representative subset of the C228T and C250T mutations (n = 59) were somatic, as evidenced by their absence in normal tissues of the patients containing the mutations in their tumors.

The 1,230 tumors represented 60 tumor types. In 26 of these tumor types, at least 15 individual tumors were evaluated (comprising a total of 1,943 individual tumors) (Fig. 1). In the remaining tumor types, only a small number of samples (2–12) was available, in part because these tumor types are generally uncommon in Western populations (Table 1). Among the tumor types in which at least 15 individual tumors were available for study, a clear distinction could be made. Eighteen of these tumor types had only occasional TERT promoter mutations (zero to three mutations, comprising 0–15% of the tumors of each type) (Fig. 1). We classified these tumor types as TERT-low (TERT-L), because they had a low frequency of TERT promoter mutations. Eight other tumor types were classified as TERT-high (TERT-H) because of their relatively high prevalence of TERT promoter mutations (16–83% of the tumors of each type).

The TERT-L tumor types included some of the most prevalent cancers, including epithelial tumors of the breast, prostate, thyroid, pancreas, gall bladder, uterus, and colon (as well as tumors of the lung, stomach, and esophagus based on prior studies) (14, 15) and leukemias. In fact, no TERT mutations were identified in any specimen of 30 tumor types that we studied, comprising a total of 546 tumors (Table 1). Some nonepithelial cancers, such as synovial sarcomas, chordomas, neuroblastomas, osteosarcomas, and ependymomas, were also TERT-L.

Eight TERT-H tumor types were identified (in addition to the previously described melanomas) (14, 15). These tumors included tumors of the CNS, transitional cell carcinomas of the urinary tract, hepatocellular carcinomas, myoid liposarcomas, and oral cavity carcinomas. Although only a small number of TERT-H tumors (other than melanomas) were examined in previous studies (15), mutations in gliomas, hepatocellular, and oral cavity carcinomas were detected, which would be expected on the basis of the high frequency of mutation in these tumor types (Table 1).

Table 1. Frequency of TERT promoter mutations

<table>
<thead>
<tr>
<th>Tumor type*</th>
<th>No. tumors</th>
<th>No. tumors mutated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondrosarcoma</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Dysembryoplastic neuroepithelial tumor</td>
<td>3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>19</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>36</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Glioma†</td>
<td>223</td>
<td>114 (51.1)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>61</td>
<td>27 (44.2)</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>91</td>
<td>19 (20.8)</td>
</tr>
<tr>
<td>Myxofibrosarcoma</td>
<td>10</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
<td>24</td>
<td>19 (79.1)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>22</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>23</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Ovarian, clear cell carcinoma</td>
<td>12</td>
<td>2 (16.6)</td>
</tr>
<tr>
<td>Ovarian, low grade serous</td>
<td>8</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Solitary fibrous tumor (SFT)</td>
<td>10</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Squamous cell carcinoma of head and neck</td>
<td>70</td>
<td>12 (17.1)</td>
</tr>
<tr>
<td>Squamous cell carcinoma of the cervix</td>
<td>22</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Squamous cell carcinoma of the skin</td>
<td>5</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Urothelial carcinoma of bladder</td>
<td>21</td>
<td>14 (66.6)</td>
</tr>
<tr>
<td>Urothelial carcinoma of upper urinary epithelium</td>
<td>19</td>
<td>9 (47.3)</td>
</tr>
</tbody>
</table>

†No mutations were found in acute myeloid leukemia (n = 48), alveolar rhabdomyosarcoma (n = 7), atypical lipomatous tumor (n = 10), breast carcinoma (n = 88), cholangiosarcoma (n = 28), central/conventional chondrosarcoma (n = 9), chronic lymphoid leukemia (n = 15), chronic myeloid leukemia (n = 6), colorectal adenocarcinoma (n = 22), embryonal rhabdomyosarcoma (n = 8), esthesioneuroblastoma (n = 11), extraskeletal myxoid chondrosarcoma (n = 3), fibromollcellar carcinoma of the liver (n = 12), gall bladder carcinoma (n = 10), gastrointestinal stromal tumor (n = 9), hepatoblastoma (n = 3), leiomyosarcoma (n = 3), conventional lipoma (n = 8), low grade fibromyxoid sarcoma (n = 9), malignant peripheral nerve sheath tumor (n = 3), medullary thyroid carcinoma (n = 24), meningioma (n = 20), mesothelioma (n = 4), pancreatic acinar carcinoma (n = 25), pancreatic ductal adenocarcinoma (n = 24), pancreatic neuroendocrine tumor (n = 68), prostate carcinoma (n = 34), spinal ependymoma (n = 9), synovial sarcoma (n = 16), or undifferentiated pleomorphic soft tissue sarcoma (n = 10) samples.

*No mutations were found in acute myeloid leukemia (n = 48), alveolar rhabdomyosarcoma (n = 7), atypical lipomatous tumor (n = 10), breast carcinoma (n = 88), cholangiosarcoma (n = 28), central/conventional chondrosarcoma (n = 9), chronic lymphoid leukemia (n = 15), chronic myeloid leukemia (n = 6), colorectal adenocarcinoma (n = 22), embryonal rhabdomyosarcoma (n = 8), esthesioneuroblastoma (n = 11), extraskeletal myxoid chondrosarcoma (n = 3), fibromollcellar carcinoma of the liver (n = 12), gall bladder carcinoma (n = 10), gastrointestinal stromal tumor (n = 9), hepatoblastoma (n = 3), leiomyosarcoma (n = 3), conventional lipoma (n = 8), low grade fibromyxoid sarcoma (n = 9), malignant peripheral nerve sheath tumor (n = 3), medullary thyroid carcinoma (n = 24), meningioma (n = 20), mesothelioma (n = 4), pancreatic acinar carcinoma (n = 25), pancreatic ductal adenocarcinoma (n = 24), pancreatic neuroendocrine tumor (n = 68), prostate carcinoma (n = 34), spinal ependymoma (n = 9), synovial sarcoma (n = 16), or undifferentiated pleomorphic soft tissue sarcoma (n = 10) samples.

†Glioma comprises 11 subtypes; see Dataset S1 and Table S3.
Clinical and Molecular Correlations in TERT-H Tumors. Sarcomas. One of the highest frequencies of TERT promoter mutation was found in myxoid liposarcoma (19 of 24 tumors, 79% with mutation). Myxoid liposarcomas account for more than one-third of all liposarcomas and ~10% of all adult soft tissue sarcomas (16). Patients are relatively young, with a peak age range between 30 and 50 y. At the genetic level, the most characteristic change is a t(12;16) (q13;p11) chromosomal translocation that results in the fusion of the FUS and DDIT3 genes (16, 17). The cellular origin of these tumors is unknown, but preadipocytic progenitor cells and mesenchymal stem cells have been implicated (18); after embryogenesis, the mitotic activity of these cells is thought to be low. Other sarcomas, also thought to originate from mesenchymal cells that do not self-renew in the absence of damage, were not TERT-H (Table 1). These sarcomas included synovial sarcomas (6% of 16 tumors) and osteosarcomas (4.3% of 23 tumors). Of note, myxoid liposarcomas have been previously shown to have a relatively high prevalence of ALT (24% of 38 tumors) (13, 19). The data, in aggregate, are compatible with the idea that myxoid liposarcomas almost always genetically activate telomere maintenance genes through either TERT promoter mutations or ALT. Hepatocellular carcinomas. Hepatocellular carcinomas (HCCs) are the third leading cause of cancer mortality worldwide, and their incidence is increasing in the United States (20). Most HCCs in the United States are associated with Hepatitis B or C Virus infection, whereas others are associated with alcoholic cirrhosis; 44% of HCC samples that we evaluated harbored TERT promoter mutations (27/61). This finding makes TERT the most commonly mutated gene yet observed in this tumor type (21, 22). The mutations seemed to occur relatively early in tumorigenesis, because they were observed in 39% of stage I well-differentiated HCCs (Table S1). TERT mutations were observed in virally associated tumors as well as cases without any underlying liver disease at similar frequencies (Table S1). There was also no difference in the prevalence of TERT promoter mutations with respect to sex, age, or ethnicity (Table S1). ALT has been observed in 7% of 121 HCCs studied previously (13).

Urinary tract cancers. Urothelial carcinoma of the bladder is the fourth most common type of cancer in American males. In 2013, over 73,000 patients will be diagnosed with bladder cancer leading to approximately 15,000 deaths in the US alone (23). Two-thirds of the 21 urothelial carcinomas of the bladder that we studied harbored TERT promoter mutations. We were also able to evaluate 19 urothelial carcinomas of the upper urinary tract, a much less common anatomic site for this histopathologic subtype of tumor. Nine of nineteen upper urinary tract urothelial carcinomas harbored TERT mutations. TERT mutations are, therefore, the most frequently mutated genes yet identified in urothelial carcinoma of either the bladder or upper urinary tract (24). The prevalence of ALT in bladder cancers is very low (1% of 188 cancers) (13).

Head and neck cancers. Head and neck cancers are almost always squamous cell carcinomas and can occur throughout the oral cavity lining (mucous membranes of the cheek, hard and soft palate, tongue, supraglottis, etc.). It is the sixth most common cancer in the world, and 50,000 cases occurred in the United States in 2012. We identified TERT promoter mutations in 17% of 70 oral cavity cancers that we evaluated. However, the anatomic distribution of the cases with TERT promoter mutations was striking: 11 of 12 cancers with TERT promoter mutations were in the oral tongue, although only 23 of 70 total cases originated in the oral tongue (P < 0.0001, Fisher exact probability test, two-tailed) (Table S2). The basis for this extraordinary selectivity is curious given the shared characteristics of the squamous epithelium lining the tongue and other parts of the head and neck, including the oral cavity. Moreover, we evaluated 22 squamous cell carcinomas of another site (the cervix) and found only one TERT mutation (4.5%) (Table 1). Most cervical squamous cell carcinomas and a subset of head and neck squamous cell carcinomas are caused by human papillomavirus, which can activate telomerase by expressing E6 and E7 viral oncogenes (25). These findings raise the possibility that human papillomavirus infection and TERT mutation may be alternative mechanisms to activate telomerase among squamous cell carcinomas. We were unable to test correlations between TERT promoter mutations and HPV status or other clinical parameters because of the small number of patients with available data (Table S2). There have been no ALT cases identified among 70 head and neck cancers, including 41 oral cavity cancers (13).

Medulloblastomas. Medulloblastoma is the most common malignant brain tumor of childhood (26). TERT mutations occurred in 21% of 91 medulloblastomas that we evaluated. As with the oral cavity cancers, TERT mutations were not distributed randomly among the medulloblastoma patients. Although medulloblastomas with TERT mutations were diagnosed at a considerably older age (median = 6 vs. 16 y, P = 0.0012, t test assuming unequal variances, two-tailed) (Fig. S1A). This observation has important implications for understanding the basis for the selectivity of the tumor types harboring TERT promoter mutations (Discussion); 45 of 90 patients had been assessed previously for orthodenticle homeobox 2 (OTX2) gene amplification and expression, and alterations in this transcription factor are known to correlate with clinically distinct molecular subtypes of medulloblastoma (27). OTX2 expression was >100-fold higher in medulloblastoma patients without TERT promoter mutations than in those patients with TERT promoter mutations (note the log scale in Fig. S1B). The high levels of OTX2 expression were usually the result of OTX2 gene amplification (Fig. S1C). The association of TERT promoter mutations with an older age at diagnosis and a lack of OTX2 overexpression raises the possibility that TERT mutations occur in a specific clinical and molecular subtype of medulloblastoma. The most likely molecular subtype of medulloblastoma that may be enriched for TERT mutations is the noninfant sonic hedgehog subtype, which is characterized by an older age at diagnosis and lower expression of OTX2 (28, 29). Larger studies will be needed to make
Conversely, no association was identified between TERT mutation and either TP53 mutation or CDKN2A deletion. Importantly, the frequency of TERT promoter mutations was considerably less in primary GBMs of pediatric patients (11% of 19 tumors) than adult patients (Discussion) (Table S3). ALT was observed in 11% of 105 adult GBM and 44% of pediatric GBM (i.e., the reverse of the pattern observed for TERT promoter mutations) (13). Primary GBM patients without TERT mutations survived considerably longer, on average, than patients with such mutations (median = 27 vs. 14 mo, P = 0.01 by the log rank test) (Fig. S3).

Astrocytomas. Infiltrative astrocytic tumors frequently progress, with recurrent lesions often of higher grade than the original lesions excised at surgery. They are most often grade II or III but can progress to grade IV (at which point they are often termed secondary GBMs). Astrocytomas of any stage rarely contained TERT promoter mutations (10% of 40 total samples) (Table S3). Instead, they more frequently contained isocitrate dehydrogenase 1 (IDH1) or isocitrate dehydrogenase 2 (IDH2) mutations (75% of 40 tumors), ATRX mutations (70% of 40 tumors), and TP53 mutations (73% of 40 tumors) (Fig. 2B). ALT has been observed in 7% of 57 astrocytomas, consistent with the high prevalence of ATRX mutations (13). The lack of activating TERT mutations in IDH1 mutant tumors is also corroborated by the lack of TERT mRNA and telomerase activity observed in these lesions (33).

Oligodendrogliomas. As their name implies, these tumors are mixed, with histologic features of both oligodendrogliomas and astrocytomas. This mixture, in part, reflects the difficulties in distinguishing the various glioma subtypes from one another on the basis of histopathologic or clinical criteria (37). The genetic features of this tumor subtype reflect this mixture: the prevalence of TERT promoter mutations (25% of 24 tumors) was intermediate between oligodendrogliomas and astrocytomas, as were the frequencies of chromosome (Chr) 1p/19q losses (34–36). Accordingly, 78% of 45 oligodendrogliomas contained chromosome arm 1p or 19q losses of heterozygosity (Fig. 2C) (34–36). Moreover, nearly all of them contained IDH1 or IDH2 mutations (93%).

ALT Vs. TERT. ALT has been observed in tumors of the CNS (particularly gliomas) more frequently than tumors of any other tissue type. Given that TERT promoter mutations are also common in gliomas, the relationship between these two features could be determined with high confidence. The tumors depicted in Fig. 2 had previously been evaluated for alterations in ATRX, which is a nearly perfect surrogate for the ALT phenotype (11, 37). Our data show that there were 50 gliomas with ATRX mutations and 83 gliomas with TERT mutations; 0 of 83 tumors with TERT mutations contained ATRX mutations (P < 0.0001, Fisher exact probability test, two-tailed).

Fig. 2. Mutations of selected genes in glioma subtypes. (A) Distribution of TERT mutations and other genetic events in 51 primary GBMs. (B) Distribution of TERT mutations and other genetic events among 40 astrocytomas, including grades II–III astrocytomas and grade IV secondary GBMs. (C) Distribution of TERT mutations and other genetic events among 45 oligodendrogliomas. (D) Distribution of TERT mutations and other genetic events among 24 oligoastrocytomas. World Health Organization tumor grade is indicated under each column. Light gray cells denote WT status in tumors.
Discussion
The results described above, as well as the results published in refs. 14 and 15, provide evidence that supports one of the hypotheses raised in the Introduction and refutes others. The first of these hypotheses was that TERT mutations would only be observed in tumors derived from tissues that are not constantly self-renewing. This hypothesis was supported in part: the vast majority of TERT promoter mutations occurred in tumors derived from tissues that do not continually self-renew. The TERT-H tumor types include only melanomas, certain subtypes of glioma, medulloblastomas, squamous cell cancers of the tongue, liposarcomas, HCCs, and urinary tract cancers. The normal transitional cells of the urinary tract have very low proliferative indices (0.64% ± 0.52%), much lower than indices of gastrointestinal tract, bone marrow, or skin (38). Normal hepatocytes also do not turnover often (39), and glial cells are thought to have limited capacity for self-renewal (40).

Two other observations also support the hypothesis. Pediatric primary GBMs rarely contained TERT mutations (11%), whereas adult primary GBMs frequently did (83%). Pediatric GBMs are presumably derived from cells that are still dividing at the time of tumor initiation, and therefore, there is no selective advantage conferred by activating telomerase through a genetic mutation. Adult GBMs, in contrast, are presumably derived from postmitotic cells, and they should require telomerase activation. Similarly, medulloblastomas are embryonal tumors that typically arise from precursor cells with high self-renewal rates that do not usually persist in adults. This finding is consistent with our observation that the mean age of medulloblastoma patients with TERT mutations was considerably older than the mean age of medulloblastoma patients without TERT mutations (Fig. S1A).

There are, however, exceptions that belie the hypothesis that TERT mutations occur only in non–self-renewing tissues. The epithelium that lines the tongue constantly self-renews, but many squamous carcinomas of the tongue harbor TERT mutations (Table S2). Additionally, the squamous epithelia of the tongue certainly would not be expected to self-renew less than other squamous epithelia of the oral cavity, but the latter rarely harbored TERT mutations (Table S2). This finding may suggest that squamous carcinomas of the tongue originate from a different cell of origin than other oral cavity squamous carcinomas. Conversely, only a subset of the tumor types derived from non–self-renewing tissues was TERT-H. For example, the TERT-H tumors included myoid liposarcomas but not synovial sarcomas. Moreover, cells of the pancreas (the islets of Langerhans and the ductal epithelial cells) rarely renew, but pancreatic tumors of all types (pancreatic neuroendocrine tumors, acinar carcinomas, and pancreatic ductal adenocarcinomas) were all TERT-L. The most we can conclude at present is that non–self-renewing cell types are the major sources of TERT-H tumors but that nonself-renewal is only one of the factors that determines whether tumor cells with TERT promoter mutations will have a selective growth advantage over adjoining cells.

The first corollary to the hypothesis raised in the Introduction was that tumor types that displayed ALT would be those types that harbored TERT promoter mutations. This corollary is soundly refuted by these data, at least in general terms. Although tumor types of the CNS and liposarcomas had high frequencies of ALT as well as high frequencies of TERT promoter mutations, these tumor types were the exceptions rather than the rule. For example, pancreatic neuroendocrine tumors have very high frequencies of ALT but no evidence of TERT mutations. Conversely, bladder cancers frequently have TERT mutations but never have ALT (13). Additionally, even among gliomas, pediatric GBMs have high frequencies of ALT and low frequencies of TERT mutations, whereas adult GBMs have the reverse pattern.

The second corollary was that the selective advantage afforded by TERT mutation would be equivalent to the advantage afforded by ATRX mutation (conferring ALT). This hypothesis was most effectively tested in gliomas, in which both ATRX coding and TERT promoter mutations were common. There was a striking mutual exclusivity with respect to ATRX and TERT mutations (P < 0.0001), lending strong support to this idea.

These results also raise many unanswered questions. In some tumor types, such as gliomas, we can imagine that all tumors have genetically acquired telomerase maintenance programs through mutations in either TERT or ATRX. However, in other tumor types with frequent ATRX mutations, such as pancreatic neuroendocrine tumors, what is responsible for activating telomerase in the fraction of cases not exhibiting ALT if it is not a mutation in the TERT promoter? Similarly, what is responsible for activating telomerase in those tumors derived from non–self-renewing cell types in which neither ALT nor TERT mutations is frequently observed, such as synovial sarcomas or osteosarcomas? Also, there are occasional individual tumors among the TERT-L types that have TERT promoter mutations (e.g., cervical cancers, ovarian cancers, and in ref. 15, lung cancers). What distinguishes these occasional cancers from others of the same histopathologic subtype? Whole-genome sequencing studies, rather than those studies limited to the exome, might provide answers to these questions.

The results recorded here have practical as well as basic scientific implications. Two-thirds of bladder cancers had TERT promoter mutations, making it the most commonly mutated gene yet identified in invasive urothelial carcinoma of the bladder. Given the persistently high mortality rate despite multimodality treatment in this group of patients, these mutations represent ideal urinary biomarkers to detect bladder cancers at an early stage and to follow patients for evidence of progression or recurrence once they have been diagnosed (41). Similarly, the high prevalence of TERT promoter mutations in HCCs and glioma subtypes provides excellent candidate biomarkers for early detection (HCC) or monitoring (HCC in the plasma and gliomas in the cerebrospinal fluid) (42, 43).

Another practical implication involves diagnostics. We conjecture that tumors with TERT promoter or ATRX mutations are derived from different precursor cells and that either type of precursor cell is different from those types that are the precursors of tumors without such mutations. This distinction could aid classification of the tumors in clinically meaningful ways. For example, Fig. 2 and Fig. S2 outline the major genetic alterations occurring in the three common types of gliomas. On the basis of the data in Fig. 2 A–C, we speculate that oligodendrogliomas that lack TERT mutations but contain ATRX mutations may behave more like astrocytomas than oligodendrogliomas and vice versa. Similarly, the primary GBMs without TERT mutations (15% of the total) may behave more like advanced progressive astrocytomas, which generally lack TERT mutations. This possibility is supported by the observation that those primary GBM patients without TERT mutations had a longer survival, on average, than other primary GBM patients (Fig. S3).

Methods
All clinical information and tissue were obtained with consent and Institutional Review Board approval from the various institutions donating material to this study, and they were obtained in accordance with the Health Insurance Portability and Accountability Act. Tissue sections were reviewed by board-certified pathologists to ensure that ≥50% of the cells used for DNA purification were neoplastic and confirm histopathological diagnosis. Oligonucleotides with the sequences 5′-M13-CCGGCGATACGACCCTCT-3′ and 5′-AGACCTGGCGTATTGGAT-3′, where M13 is a universal sequencing primer site with sequence 5′-tgtaaaagccgacagt-3′, were used to PCR-amplify the proximal TERT promoter containing C228 and C250 (chr5: 1,295,228; chr5: 1,295,250, respectively; hg19) for Sanger sequencing using standard methods (44). Primary GBM copy number data as well as ALT status were derived from the data published in refs. 37, 45, and 46, and OTX2 copy number expression was derived from the data published in ref. 27. Brain tumor patients were treated at the Tisch Brain Tumor Center at Duke. For the purposes of this study, secondary GBM designates a GBM that was resected and from which a second glioma specimen was obtained for analysis. The majority of secondary GBM specimens had a diagnosis of a lower-grade glioma (grades I–III), and all other GBMs were considered to be primary GBMs. Pediatric GBM samples were defined as those samples occurring before 21 y of age.
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