A Novel Telomere Structure in a Human Alternative Lengthening of Telomeres Cell Line

Robert A. Marciniak,1,2 David Cavazos,1 Richard Montellano,1 Qiujun Chen,1 Leonard Guarente,3 and F. Brad Johnson1

1Department of Medicine, University of Texas Health Science Center at San Antonio, 2South Texas Veterans Health Care System, San Antonio, Texas; 3Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts; and 4Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract

Cancer cells require mechanisms to maintain telomeres. Most use telomerase, but 5% to 20% of tumors use alternative lengthening of telomeres (ALT), a telomerase-independent mechanism that seems to depend on recombination. ALT is characterized by amplification of telomere TTAGGG repeats to lengths beyond 50 kb, by elevated rates of telomere recombination, and by nuclear structures called ALT-associated promyelocytic leukemia bodies. In Saccharomyces cerevisiae, survivors of telomerase inactivation also use recombination to maintain telomeres. There are two types of survivors, which differ in telomere structure. The first possesses telomere repeats and the Y' subtelomeric element amplified together as a tandem array at chromosome termini (type I), and the other possesses amplification of telomeric repeats alone (type II), similar to previously described human ALT cells. Here, we describe the first human ALT cell line having "tandem array" telomeres with a structure similar to that of type I yeast survivors. The chromosome termini consist of a repeat unit containing ~2.5 kb of SV40 DNA and a variable amount of TTAGGG sequence repeated in tandem in an average of 10 to 20 times. Similar to previously described ALT cells, they show evidence of telomere recombination, but unlike standard ALT cells, they lack ALT-associated promyelocytic leukemia bodies and their telomeres are transcribed. These findings have implications for the pathogenesis and diagnosis of cancer. (Cancer Res 2005; 65(7): 2730-7)

Introduction

Most immortalized cancer cells use telomerase to maintain telomeres through repeated rounds of cell division, but some cancer cells instead use alternative lengthening of telomeres (ALT; refs. 1–3). ALT is particularly common in certain sarcomas, including osteosarcomas, and in glioblastoma multiforme (4–6). The type of telomere maintenance mechanism is a strong prognostic indicator in glioblastoma multiforme (5) but not in osteosarcoma (4). Furthermore, it is anticipated that ALT cancers will not be responsive to telomerase inhibitors and that the use of such inhibitors may select for the emergence of ALT in tumors (7). The ALT mechanism is not fully understood, but seems to involve telomere recombination (7–10) and is characterized by telomeres of heterogeneous length that have size distributions that extend from <2 to >20 to 80 kb (3, 11). ALT cells are also characterized by ALT-associated promyelocytic leukemia bodies (APBs), which are nuclear structures containing telomere DNA, promyelocytic leukemia (PML) protein, TTAGGG repeat binding factors 1 (TRF1), and 2 (TRF2), and a variety of recombination proteins including RAD51 and the Werner and Bloom syndrome proteins (3, 12–14).

In Saccharomyces cerevisiae, inactivation of telomerase causes most cells to die from telomere dysfunction, but rare cells survive and maintain telomeres by recombination (15). There are two survivor classes, types I and II, that are distinguished by their genetic requirements and telomere structures. Type I survivors depend on RAD51 and have telomeres comprising a tandem array of amplified units consisting of Y' subtelomeric elements and telomere repeats, whereas type II survivors depend on RAD50 and SGS1, the yeast homologue of the Werner and Bloom syndrome helicase genes, and have amplification primarily of telomere repeats alone (14, 16–18). All human ALT cells thus far described have a telomere structure similar to yeast type II survivors. Here, we describe a novel human ALT cell line with a structure similar to yeast type I survivors.

Materials and Methods

Cell culture. Cell lines AG00780 (Werner mutant primary fibroblast), AG11995 (SV40-transformed Werner mutant fibroblast) and W138 75.1 (SV40-transformed fetal lung fibroblast, AG07217) were obtained from the Coriell Cell Repository (Camden, NJ). HeLa (human cervical carcinoma, catalog number CCL-2) cells were obtained from the American Type Culture Collection (Manassas, VA). WV (SV40-transformed Werner mutant fibroblast; ref. 19) was kindly provided by Prof. Sydney Shall (Cell and Molecular Biology Laboratory, University of Sussex, Sussex, United Kingdom). Cells were grown in 5% CO2 under conditions recommended by the supplier.

Telomerase enzymatic assays. Telomerase enzymatic activity was detected using the TRAPEze telomerase detection kit (Chemicon, Temecula, CA). Fluorescently labeled products were detected using a Typhoon imager (Amersham, Piscataway, NJ).

Quantitative PCR for telomerase reverse transcriptase mRNA. Total cellular RNA was prepared using the RNeasy mini kit (Qiagen, Valencia, CA) and cDNA was prepared using the high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). Human telomerase reverse transcriptase (hTERT) mRNA was detected using a Taqman-MGB probe (Applied Biosystems, Foster City, CA). Primers used were 5'-GACATGGA- GAACAAGCTGTTTGC-3', 5'-AACAAGAATTCAATCCACAAACG-3', and 5'-ATTCGGCGGGACGGG-3' (Taqman-MGB probe, FITC-conjugated), which flank the exon 9/10 boundary of the hTERT cDNA. Amplification conditions were 30 seconds at 95°C and 60 seconds at 60°C in a Mx3000P thermal cycler (Stratagene, La Jolla, CA). Glyceraldehyde-3-phosphate dehydrogenase mRNA levels were measured under the same conditions as an internal control (Assays on Demand, Applied Biosystems). The amplification efficiency of the hTERT primers was 96%.

Requests for reprints: F. Brad Johnson, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, 405A Stellar Chance Labs, 6317; E-mail: johnsonb@mail.med.upenn.edu or Robert A. Marciniak, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

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Telomere Southern blots. Total genomic DNA was prepared using the Blood & Cell Culture DNA Mini Kit (QIAGEN). Total DNA (2 to 5 μg) were digested with the indicated endonucleases and resolved on a 0.5% agarose gel. 32P-d(CCCTAA)3 probe was prepared using polynucleotide kinase. For terminal restriction fragment (TRF) analyses (Fig. 1), gels were dried and hybridization done in the gel as described (20). For standard Southern blots (Fig. 3), nucleic acids were transferred to a GeneScreen Plus membrane (Perkin-Elmer, Boston, MA) using a PosiBlot Pressure Blotter apparatus (Stratagene), and hybridized to probe. The weighted average of the hybridization signal was used to determine mean telomere length: $\Sigma$ (OD, $x$ length) / $\Sigma$ (OD). For two-dimensional Southern blots, all procedures were done as described (21) using 10 μg of total cellular DNA digested with BamHI, BglII, EcoRI, and XbaI. For SV40 hybridization, SV40 PCR product (nucleotides 232-654) was 32P-labeled by random priming, hybridized, and washed per standard protocols as described (22). Quantitation of Southern blots was done with Imagequant software analysis of phosphoimager scans and using average local background subtraction.

Cloning of telomere repeat. To isolate terminal array DNA from most genomic sequences, genomic DNA from cell line AG11395 was digested with BamHI, BglII, EcoRI, SacI, and XbaI (which do not cleave within the terminal array), the products were resolved on 0.5% agarose gel, and DNA at limit mobility was isolated. This DNA was then cut with SphI (which was predicted to cut once within the array), and the fragments were then ligated to double-strand oligonucleotide P1-SphI (5'-TGGCCCGCACCCTTTAATT-TACCGATG-3' and 5'-CGTAAATTAAGGTGGCGGGGT-3'). Unligated oligos were removed by filtration through Sephadex G25 (Pharmacia). Ligation products were then linearly amplified with Platinum Taq (Invitrogen, Carlsbad, CA; 30 cycles, 56°C for 45 seconds, 72°C for 5 minutes, and 94°C for 45 seconds) using primer P2-TEL [5'-GCACTTCAGCTGCGCCAT-GAGG(CCCTAA)$_i$-3'], which introduced a second unique primer site for subsequent "nested" PCR. Finally, the target DNA was exponentially amplified using primers P1 (5'-TGGCCCGCACCCTTTATT-3'TACCGATG-3') and P2 (5'-ATTGACGTGCGCAATGGG-3'; 30 cycles, 56°C for 45 seconds, 72°C for 5 minutes, and 94°C for 45 seconds). The products of this reaction were cloned into T-vector (Promega, Madison, WI), and sequenced. Once the identity of the sequence integrated at telomeres was determined, additional PCR clones were obtained and sequenced by direct PCR from AG11395 genomic DNA using SV40-specific primers and P2-TEL and primer P1-TEL [5'-GCTGGCCGGCACCCCTTTAATTA(TTAGGG)$_i$-3'].

Fluorescence in situ hybridization. For telomere repeat fluorescence in situ hybridization (FISH), a Cy3-conjugated peptide nucleic acid (PNA) probe [[(CCCTAA)$_i$; Applied Biosystems] was used as described (23). For

Figure 1. An unusual telomere structure in an immortalized Werner syndrome mutant cell line. Cells included WI38 75.1 (telomerase-negative, SV40-immortalized human fibroblasts), HeLa (telomerase-positive cervical carcinoma cells), AG00780 (Werner syndrome primary fibroblasts), and AG11395 (SV40-immortalized derivative of AG00780). A, TRF length analysis; 2 μg of genomic DNA from each of the cell lines indicated were digested with HindIII and RsaI, resolved on a 0.5% agarose gel and the products analyzed by in-gel hybridization using a d(CCCTAA)$_i$ probe. The mean TRF lengths of AG00780 and AG11395 are 8 and 2.6 kb, respectively; B, TRAP performed on the indicated amounts of cell extracts. The lowermost band (IC) is an internal PCR control. The absence of TRAP inhibitors was assessed by mixing 100 ng each of HeLa and AG11395 extracts; C, quantitative reverse transcriptase-PCR analysis of hTERT mRNA levels relative to HeLa cells. Columns, mean; bars, ± SD of three independent measurements; D, comparison of TRF lengths produced by restriction endonucleases with six-base recognition sequences. Genomic DNA (2 μg) from the indicated cell lines was digested as indicated and the products analyzed by in-gel hybridization as in (A).
SV40 FISH, an equimolar mixture of probes was prepared from plasmid pB5SV40 (American Type Culture Collection: a 2,214 bp Nco I fragment, and 1,935 and 1,466 bp Pvu II fragments. These fragments overlap the T antigen coding sequence and the SV40 regulatory region. After gel purification, the fragments were labeled with biotin using the NEBlot Phototote Kit (New England Biolabs, Beverly, MA). Slide preparation, hybridization, and detection were done following standard protocols (22). RNase pretreatment of mitotic spreads was done after fixation onto microscope slides by incubation for 30 minutes at 37 °C with 20 ng RNase A (Invitrogen) diluted in 40 mL of PBS. Slides were then washed thrice for 2 minutes in PBS, and processed for PNA-FISH as described above.

Indirect immunofluorescence. Fixation and indirect immunofluorescence were done as described (24). Antibodies used and dilutions were mouse monoclonal anti-PML (1:100; Santa Cruz Biotech, Santa Cruz, CA), affinity purified rabbit anti-TRF1 (1:100; Alpha Diagnostic, San Antonio, TX), Cy3-conjugated donkey anti-rabbit IgG (1:300; Amersham Biosciences, Piscataway, NJ) and FITC-conjugated donkey anti-mouse IgG (1:100; Vector Labs, Burlington, CA).

Dot blot of telomere RNAs. Total RNA from the indicated cell lines was prepared as described above, and concentration was determined by absorbance at 260 nm. RNA was blotted onto GeneScreen Plus (Perkin-Elmer). Hybridization was done with biotinylated d(CCTAATA)3 or d(TTAGGG)3 probes (IDT, Coralville, IA), and probe detected using the Detector horseradish peroxidase chemiluminescent blotting kit (KPL, Gaithersburg, MD). Blots were treated with blocking buffer and then hybridized to probe for 6 hours at 37 °C. The presence of telomere RNAs was also verified by qPCR on cDNA prepared cell lines, using conditions described previously (25).

Results

Immortalized AG11395 cells have unusually short telomere TTAGGG repeat binding factors and lack telomerase activity. As part of an analysis of genetic requirements for telomere maintenance in human cells, we found that an SV40-transformed, immortalized cell line (AG11395, ref. 26), which lacks the Werner syndrome helicase (WRN, ref. 24), seemed to have unusually short telomeres. Standard TRF analysis, which involves a Southern blot of telomeric DNA, digested with enzymes (Fig. 1D). As predicted, some enzymes yielded long ALT-like TRF lengths (e.g., Afl I in Fig. 1D). The TRF length of the parental AG00780 line digested with Afl I was not longer than those produced by other endonucleases (Fig. 1D), indicating that the increased TRF length of AG11395 was not due to scarce subtelomeric Afl I sites. Furthermore, the pattern of TRF fragments is remarkably similar for several enzymes (e.g., Sph I, Nde I, and Eco RV), consistent with these enzymes having a single recognition site within each unit of the array; the varying size of the fragments could be due to heterogeneity in the lengths of telomere repeat or non–telomere repeat components of each unit, or both.

SV40 sequences are associated with telomere repeat DNA in AG11395. We cloned the sequences associated with the telomere repeats using the strategy outlined in Fig. 2A (also see Materials and Methods). Briefly, telomere DNA was separated from bulk genomic DNA, digested with Sph I (predicted to cut once within each array unit), ligated to adapters and amplified by PCR using adapter-specific and telomere repeat-specific primers. This procedure produced a smear of products, including two predominant products at 1.4 and 1.7 kb (Fig. 2B), which were then cloned. These clones yielded information on the nature of the non–telomere repeat sequences and the centromeric junction between these sequences and telomere repeat sequences. This information was used to design primers to amplify and clone fragments containing the telomeric junction. Sequencing of six clones containing the centromeric junction and five clones containing the telomeric junction revealed that the telomere repeat sequences in each unit are interrupted by a fragment of the SV40 viral genome that extended from nucleotide 1696 through nucleotide 0/5243 and continued to nucleotide 4469 of the circular SV40 genome, abbreviated SV40 4469 to 1696 (Fig. 2C). The centromeric and telomeric junction sequences were the same in all clones analyzed. The SV40 fragment contains the regulatory region, which includes the origin of replication and the early and late promoter sequences (Fig. 2D). Degenerate telomere repeats (e.g., TTAGGG) were found within the first 45 nucleotides flanking the SV40 DNA, but the remaining telomeric repeats matched the standard TTAGGG repeat. The fact that all clones had the same SV40-telomere repeat junctions indicates that a single SV40 integration event had occurred, followed by propagation along a chromosome and between chromosomes. Although the SV40 sequence did not contain an intact large T antigen coding sequence, large T antigen was present on immunoblot (data not shown), indicating that the original SV40 sequences used to transform the parental cell line were extant elsewhere in the genome.

The majority of telomere repeats and SV40 sequences are closely associated in AG11395. The association of the majority of
SV40 sequences with telomere repeats was also shown by Southern blot (Fig. 3A). Endonucleases that do not cut within the array units yielded long, heterogeneous TRFs that had identical or nearly identical patterns of hybridization with SV40 and telomere probes; those enzymes that cut within the unit but do not cut both 5′ and 3′ of the SV40 hybridization target also showed nearly identical patterns of hybridization to both probes (cf. SphI, EcoRV; Fig. 3A). Enzymes that cut multiple times within each unit—and cut between the location of the SV40 hybridization probe and the telomere repeats—dissociated the patterns of hybridization with the two probes. The multiple bands in the Hinfl/RsaI, DraI or HindIII digested DNA hybridized to the telomere repeat probe showed substantial heterogeneity in the amount of telomere repeat sequence within a unit repeat. In contrast, the single bands observed for these digests hybridized to an SV40 probe indicated less heterogeneity in the SV40 sequences contained within the unit repeat. Thus, the heterogeneity in array unit length seems to be caused by variation in the length of telomere repeats within each unit; in samples cut with enzymes that only cut the array unit once (e.g., SphI; Fig. 3A), the long size of some fragments indicates infrequent SV40 sequences at some arrays, whereas the decline of hybridization of the telomere repeat probe to short fragments that hybridize strongly to the SV40 probe indicates that SV40 sequences may be interrupted by very short (and possibly even no) telomere repeat DNA in other arrays. In addition, the finding that there is little heterogeneity in the size of SV40 fragments in the arrays is consistent with the constancy of sequence at the SV40-TTAGGG junctions, and indicates that a single primordial integration event of the SV40 fragment into telomere repeat DNA was followed by propagation of the SV40 sequence along and between chromosomes.

SV40 and telomere repeat sequences are found at chromosome termini in AG11395. Although the sequence and Southern analyses showed an association between SV40 and telomere repeat sequences in AG11395, they do not show where in the genome these sequences are located. PNA-FISH for telomere repeats (23) confirmed that the majority of TTAGGG repeats are near the chromosome ends and showed large inter- and intrachromosomal variation in hybridization signal in the AG11395 cell line (Fig. 3B), similar to that observed at standard ALT telomeres. FISH analysis with SV40 regulatory region probes (Fig. 3C) confirmed that the majority of SV40 hybridization signal was seen near the chromosome ends. The strength of telomere repeat and SV40 signals at
chromosome termini varied between chromosomes, and between p and q arms within the same chromosome. No SV40 hybridization signal near the chromosome ends was seen in metaphases from WI38 75.1, another SV40-transformed ALT cell line (data not shown).

AG11395 cells lack alternative lengthening of telomeres–associated promyelocytic leukemia bodies. APBs have been used as a morphologic marker of ALT because they are found only in immortalized cells that lack telomerase and that have telomeres of extremely heterogeneous length (3, 6, 12). APBs are characterized by the colocalization of the PML protein with telomere repeat binding factors, including TRF1 (3). APBs were readily detected in WI38 75.1 ALT cells, as indicated by colocalization of the PML and TRF1 proteins (Fig. 4A-C), but no such colocalization was seen in any AG11395 cell (Fig. 4D-F). Thus, AG11395 cells lack ALT-associated PML bodies.

Telomere repeat DNA is transcribed in AG11395. While analyzing metaphase spreads, we noticed that 88% of AG11395 interphase nuclei had an unusually bright and diffuse pattern of telomere PNA-probe hybridization visible on short exposures (Fig. 5A). Given a possible role for extrachromosomal circular DNAs in generating tandem array structures via a rolling-circle intermediate (3, 15, 21, 29), we first assessed whether extrachromosomal DNAs containing telomere and SV40 sequences were present in AG11395. Two-dimensional gel electrophoresis and Southern analysis showed extrachromosomal circular species containing telomere repeat and SV40 DNA in AG11395 (Fig. 5D; and data not shown). Similar species containing telomere repeat but not SV40 DNA were also present in the ALT lines WI38 75.1 and WV. All three ALT lines had higher levels of circular species than the non-ALT BJ-hTERT line. However, the level of circular species in AG11395, although the highest among the lines examined, were still <0.2% of telomere repeat DNA and so could not account for the diffuse telomere PNA staining of AG11395 nuclei. Given the presence of the SV40 promoters integrated adjacent to telomere sequences, we next assessed whether RNA transcripts containing telomere repeat sequences were present. RNase A treatment prior to hybridization to the telomere PNA probe eliminated the diffuse nuclear hybridization signal (cf. Fig. 5A and B). Telomere DNA-hybridization signals at chromosome termini remained detectable, if a standard length (i.e., longer) exposure was used to acquire the FISH signal (Fig. 5C). To confirm that the diffuse nuclear hybridization signal was due to the presence of RNA, we did dot blot hybridization of total cellular RNA isolated from this cell line (Fig. 5E). A C-strand telomere probe hybridized to immobilized total cellular RNA isolated from AG11395, but not to total cellular RNA from HeLa (telomerase-positive) or WI38 75.1 (ALT) cells. When a G-strand telomere probe was used, no hybridization signal from total cellular RNA was observed (Fig. 5E). This indicates either that the telomere repeats are transcribed only in the direction yielding G-rich transcripts, or that the C-rich transcripts, if transcribed, are not stable and do not accumulate.

Figure 3. SV40 and (TTAGGG)n repeats exist in a tandem array structure near chromosome ends in AG11395. A, evidence for the tandem array structure of telomere repeat and SV40. High molecular weight genomic (2 μg) DNA from AG11395 was digested with the enzymes indicated, and analyzed by Southern blot of a 0.5% agarose gel. Hybridized to a telomere-specific hybridization probe (left). The same blot rehybridized to an SV40 regulatory region probe (right). Enzymes used are indicated above each lane, and size standards (in kilobases) are shown. "Bam/Bgl/Eco/Xba" indicates simultaneous digestion with BamHI, BglII, EcoRI, and XbaI; B, telomere repeats are near chromosome ends in cell line AG11395. FISH to mitotic chromosomes with a Cy3-labeled PNA telomere probe (red), counterstained with 4′,6-diamidino-2-phenylindole (blue). An enlarged view is shown in the inset; C, SV40 sequences are near chromosome ends in cell line AG11395. FISH with a FITC-labeled SV40 regulatory region probe (green). An enlarged view is shown in the inset.
Discussion

The telomere structure of the AG11395 cell line, consisting of an average of 10 to 20 tandem repeats of a SV40-(TTAGGG)<500 unit at chromosome ends, is the first example of "tandem array" telomeres in human cells. A similar telomere structure was also described in a murine embryonic stem cell line in which telomerase had been inactivated through disruption of the Terc telomerase RNA template gene and the cells then passaged through crisis; in this line, the non–telomere repeat component of the array consisted of an interspersed repetitive element from the mouse genome rather than SV40 sequence (30). Both of these cases have telomeres that bear structural similarity to those of S. cerevisiae type I survivors of telomerase deletion, and they show that tandem array telomeres can stabilize chromosome termini in the absence of telomerase in mammalian cells.

In yeast lacking the Sgs1p RecQ family helicase, only type I survivors of telomerase deletion emerge (14, 18, 28). Because there is evidence that the mammalian WRN and BLM RecQ helicases participate in telomere maintenance (31–35), including standard ALT (36), it is possible that the absence of WRN in AG11395 contributed to the development of its type I-like telomeres. However, if WRN does participate in the standard ALT mechanism, there is no absolute requirement for WRN because the only other immortalized cell line lacking WRN, WV (19), has a standard ALT phenotype (Fig. 5D).5 A possible role for WRN in regulating ALT is intriguing because sarcomas account for much of the increased cancers in Werner syndrome (37) and because sarcomas are often characterized by ALT (3, 6).

Although there are differences between the tandem array telomeres of AG11395 and previously described ALT cells, there are also similarities. The presence of the same SV40 sequences in association with at least the majority of the telomere repeats indicates that telomere recombination led to the propagation of this sequence between chromosome termini. The ability of a marked telomere to propagate among different telomeres in AG11395 also

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5 R.A. Marciniak and F.B. Johnson, unpublished data.
supports this conclusion (38). Furthermore, the presence of elevated levels of extrachromosomal circular DNA containing telomere repeat and SV40 sequences suggests the possible use of such species to amplify telomere ends by rolling circle replication, as suggested previously (3, 15, 21, 29). FISH analyses of metaphase chromosomes revealed heterogeneity in the apparent length of the tandem arrays among chromosome termini, similar to standard ALT and also consistent with a recombination-based mechanism of telomere maintenance. Recombination might thus underlie both forms of ALT, and it remains to be determined if there are differences in the particular recombination pathways employed, as there are for type I and II survivors in yeast. Furthermore, it is also possible that reverse transcription of transcripts containing more than one array unit, followed by integration into an existing array, might contribute to maintenance of the arrays. 

What is the significance of the SV40 sequences in the tandem telomeres? First, their association with most or all of the telomere repeat DNA implies that the SV40 sequences provide a selective advantage in this context, although the nature of this function is unclear. It is possible that the 3′ ends of replication intermediates generated by the origin of replication provide substrates for telomere recombination. Alternatively, the origin might help maintain epissomal species containing telomere repeat and SV40 sequences and thus facilitate a rolling circle mechanism of telomere amplification; our observation that AG11395 had the highest levels of circular species among the three ALT lines that we examined is consistent with this possibility. Second, the SV40 early promoter is oriented so that it would give rise to G-rich, rather than C-rich, telomere repeat transcripts, and it is likely that it is responsible for the transcripts observed in AG11395. The transcription of telomeric sequences is remarkable because telomeres are generally transcriptionally silenced (39). However, yeast type I survivors do transcribe the subtelomeric Y′ element (40), which is silenced in normal cells. Thus, loss of telomere silencing might be a general feature of tandem array telomeres. Alternatively, the episomal telomere DNA species might have a chromatin structure permissive to transcription and thus be the source of the telomere repeat-containing transcripts. Third, the telomeric SV40 sequences are very likely derived simply from the SV40 sequences used to transform the primary cells that gave rise to AG11395 (26). Nonetheless, certain human tumors contain SV40 DNA (41) and it will be of interest to test if any of these have SV40 sequences at telomeres.

Although AG11395 is immortal and lacks detectable telomerase activity, and is thus ALT, it lacks the APBs and long telomeres in standard TRF assays characteristic of standard ALT cells. One or both of these markers of ALT are used to screen tumors for the ALT phenotype, and so the finding that nonstandard ALT exists in human cells is of importance for the diagnosis of tumor type. Although standard ALT clearly accounts for some cases of telomerase-negative tumors, there are tumors that are neither telomerase-positive nor standard ALT, for example up to half of glioblastomas (5). It will be of interest to screen such tumors for the existence of tandem array telomeres, and to determine if such a nonstandard ALT mechanism correlates with responses or outcomes to different therapies.

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References


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