Mutations in the TERC template sequence can be incorporated into the telomeres of human tumors

Radwa Sharaf, Garrett M. Frampton, Lee A. Albacker*

Foundation Medicine Inc., Cambridge, MA, United States of America

* lalbacker@foundationmedicine.com

Abstract

Telomerase-mediated lengthening is a mechanism by which some cancer cells avoid senescence-mediated cell cycle arrest due to shortened telomeres. By reverse transcribing an RNA template, encoded by TERC, the enzyme telomerase synthesizes the elongation of telomeric DNA using the 3' end of the chromosome as a primer. TERC harbors a highly conserved template region consisting of 11 nucleotides spanning hg19 coordinates chr3:169482793–169482803. In human cell lines, when TERC was mutated to alter its template region, telomerase generated the predicted mutant telomeric repeats. However, it is unknown if this can occur in human clinical samples. Here, we report on the rare occurrence of two tumor samples where TERC template mutations were reflected in telomeric repeats.

Introduction

Telomeres are protective DNA-protein complexes present at the ends of linear chromosomes, consisting of repetitive hexamers (TTAGGG) that shorten in length with every cell division by an average of 50–150 base pairs [1, 2]. If telomere length falls below a critical threshold, cells undergo senescence. In order to allow for infinite proliferation, tumor cells must overcome the telomere shortening problem [3]. Telomerase-mediated lengthening is a telomere maintenance mechanism, which relies on the overexpression of the telomerase enzyme, encoded by TERT, and was observed in 85–90% of tumors [4, 5]. By reverse transcribing TERC, which encodes an RNA template, the telomerase enzyme can synthesize the elongation of telomeric DNA using the 3’ end of the chromosome as a primer [6, 7].

TERC is a lncRNA harboring a highly conserved template region consisting of 11 nucleotides spanning hg19 coordinates chr3:169482793–169482803 encoding GTTAGGTTAG [8, 9]. Positions 1–5 basepair with the end of the telomere and positions 6–11 are reverse transcribed into new telomeric sequence [10, 11]. In human cell lines, when TERC was mutated to alter its template region, telomerase generated the predicted mutant telomeric repeats [12–14]. However, this has never been demonstrated previously in human clinical samples. Here, we report two tumor samples where TERC template mutations were reflected in telomeric repeats.
Results and discussion

Across 309,384 unique tumor samples from the Foundation Medicine dataset, we identified 120 samples carrying 137 mutations in TERC’s template sequence (Fig 1A). We observed an over-representation of mutations at chr3:169482801 (position 9), seen in 68% (82) of samples with a mutation. Overall, 88% of samples harbored only one mutation in TERC’s template region, but in 12% of samples, multiple mutations in the template sequence were observed, where one mutation always impacted chr3:169482801 (position 9) and the other mutation was either at chr3:169482800 (position 8), or chr3:169482803 (position 11), or both. Interestingly, 86% (118) of the mutations identified were a transition from the reference base to an A. Of note, the observed allele frequency of these TERC template mutations was low, with a median of 0.02 (Fig 1B), indicating that only a small proportion of tumor cells across these samples carry a TERC template mutation.

We then checked for the presence of mutated telomeric reads that matched the mutation of each sample’s TERC template sequence and found two samples that fit this criterion (Fig 1C). The allele frequency of the TERC mutation in both samples was in the top 90th percentile (0.23 and 0.16). Sample 1 had a G > T mutation at chr3:169482798 (position 6) on the positive strand, which results in a C > U mutation in the middle of TERC’s template sequence. This mutation results in the priming of the synthesis of telomeric ends with TTAGTG repeats, instead of the canonical TTAGGG repeats (Fig 1C). Sample 1 is an ovary epithelial carcinoma and had an amplification in RAD21, which we have previously shown to be associated with increased telomeric content [15]. The telomeric content measured for sample 1 was 1330.3 telomeric reads per GC-content matched million reads (TRPM), which lies at the 65th percentile across all 2623 ovary epithelial carcinoma samples in our cohort and at the 62nd percentile of RAD21-altered ovary epithelial carcinomas. Sample 2 harbored an A > G mutation at chr3:169482802 (position 10) in TERC’s template sequence, which results in the synthesis of TTGGGG repeats instead of TTAGGG (Fig 1C). Sample 2 is a skin melanoma sample and harbored a TERT promoter mutation (-124 C > T), associated with upregulation of telomerase expression. The telomeric content of sample 2 was 1281.9 TRPM, at the 64th percentile of all 5204 skin melanoma samples in our cohort and at the 67th percentile of TERTp-mutated melanomas. As shown in Fig 2, the occurrence of altered telomeric sequence was observed across entire reads, where the mutated telomere variant repeats (TVRs) accounted for 0.63% of TVRs of sample 1 and 0.56% of TVRs in sample 2. From these data, we concluded that the altered template sequences were incorporated into telomeres in these samples.

Mechanistically, positions 1–5 of the TERC template sequence must basepair with the sequence transcribed from positions 7–11 to enable further elongation. Sample 1 had a mutation at position 6, which avoids this guide/template constraint (Fig 2A). Sample 2 had an alteration at position 10, which would necessitate a G:U pairing between the mutated telomeric repeat and the unmutated position 4 of TERC’s template sequence (Fig 2B). We hypothesize that in this case, wobble-base pairing allows for binding and priming of telomeric elongation [16, 17]. We also speculate that the presence of comutations associated with increased telomerase activity (pTERT and RAD21), played a role in incorporating mutated guide sequences into telomeric repeats.

In our dataset, the prevalence of TERC template mutations was quite low (0.04%). This is consistent with the observation that the expression of mutant template RNA in cancer cell lines negatively impacts cellular survival and proliferation [14, 18, 19]. Others have shown that mutated template sequence increases the sensitivity of cancer cell lines to a variety of chemotherapeutic drugs [20]. Similarly, in Saccharomyces cerevisiae and Tetrahymena thermophila, mutations of the template sequence resulted in mutated telomeres, but negatively impacted
Mutations in the TERC template sequence can be incorporated into the telomeres of human tumors.

**Fig 1.** A. Figure depicting the positions along TERC's template sequence that were mutated in samples across Foundation Medicine's dataset. B. A histogram of the allele frequencies of the TERC template mutations detected. C. Telomeric reads detected in samples harboring WT TERC, a 169482798 G>T TERC mutation, or a 169482802 A>G TERC mutation.

https://doi.org/10.1371/journal.pone.0272707.g001
Mutations in the TERC template sequence can be incorporated into the telomeres of human tumors.
cell survival [21–23]. Our work demonstrates that mutations in the TERC template sequence exist in a small fraction of clinical tumor samples and rarely, this mutated template sequence can prime the synthesis of mutated telomeric repeats in clinical tumor samples.

**Methods**

**Ethics approval and consent to participate**

Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). Consented data that can be released is included in the paper. Institutional review board approval of the study protocol was obtained prior to study conduct and included a waiver of informed consent.

**Sample sequencing**

The Foundation Medicine dataset consisted of specimens sequenced as a part of routine clinical care using a targeted next-generation sequencing assay as previously described (FoundationOne CDx) [24, 25]. The pathologic diagnosis of each case was first made in the referring center and was then confirmed in our facility (Foundation Medicine, Cambridge MA). All samples contained a minimum of 20% tumor nuclei. The samples were assayed by adaptor ligation hybrid capture, performed for all coding exons of 309 cancer-related genes plus select introns from 34 genes frequently rearranged in cancer. Sequencing of captured libraries was performed using the Illumina sequencing platform to a mean exon coverage depth for targeted regions of >500X, and sequences were analyzed for genomic alterations by an automated pipeline.

**Telomeric reads**

For each sample, we generated a list of possible read sequences where the read exclusively consisted of TTAGGG repeats, except that the nucleotides mutated in TERC’s template were reflected in the repeat sequence.

**Acknowledgments**

We thank patients who permitted the use of their samples for research purposes.

**Author Contributions**

**Conceptualization:** Radwa Sharaf, Garrett M. Frampton, Lee A. Albacker.

**Formal analysis:** Radwa Sharaf.

**Investigation:** Radwa Sharaf, Lee A. Albacker.

**Supervision:** Garrett M. Frampton, Lee A. Albacker.

**Visualization:** Radwa Sharaf.

**Writing – original draft:** Radwa Sharaf.

**Writing – review & editing:** Radwa Sharaf, Garrett M. Frampton, Lee A. Albacker.

---

**Fig 2.** A model illustrating the impact of TERC’s template mutations on the synthesis of telomeric repeats in sample 1 harboring a 169482798 G>T mutation (A) and sample 2 harboring a 169482802 A>G (B).

https://doi.org/10.1371/journal.pone.0272707.g002
References

1. Huffman KE, Levene SD, Tesmer VM, Shay JW, Wright WE. Telomere shortening is proportional to the size of the G-rich telomeric 3’-overhang. J Biol Chem. 2000; 275:19719–22. https://doi.org/10.1074/jbc.M002843200 PMID: 10787419

2. Rahman R, Forsyth NR, Cui W. Telomeric 3’-overhang length is associated with the size of telomeres. Exp Gerontol. 2008; 43:259–65. https://doi.org/10.1016/j.exger.2008.01.005 PMID: 18280685

3. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144:646–74. https://doi.org/10.1016/j.cell.2011.02.013 PMID: 21376230

4. Kim N, Piatyszek M, Prowse K, Harley C, West M, Ho P, et al. Specific association of human telomerase activity with immortal cells and cancer. Science (80-). 1994; 266:2011–5. https://doi.org/10.1126/science.7605428 PMID: 7605428

5. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997; 33:787–91. https://doi.org/10.1016/S0959-8049(97)00062-2 PMID: 9282118

6. Morin GB. The human telomeric terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell. 1989; 59:521–9. https://doi.org/10.1016/0092-8674(89)90035-4 PMID: 2805070

7. Blackburn EH, Greider CW, Henderson E, Lee MS, Shampay J, Shippen-Lentz D. Recognition and elongation of telomeres by telomerase. Genome. 1989; 31:553–6. https://doi.org/10.1002/gene.327031043 PMID: 2875693

8. Chen J-L, Blasco MA, Greider CW. Secondary Structure of Vertebrate Telomerase RNA. Cell. 2000; 100:503–14. https://doi.org/10.1016/s0092-8674(00)80867-x PMID: 10721988

9. Naggal N, Agarwal S. Telomerase RNA processing: Implications for human health and disease. Stem Cells. 2020; 38:1532–43. https://doi.org/10.1002stem.3270 PMID: 32875693

10. Rubiosova M, Donssova O. Human Telomerase RNA: Telomerase Component or More? Biomolecules. 2020;10. https://doi.org/10.3390/biom10060873 PMID: 32517215

11. Sarek G, Marzec P, Margalef P, Boulton SJ. Molecular basis of telomere dysfunction in human genetic diseases. Nat Struct Mol Biol. 2015; 22:867–74. https://doi.org/10.1038/nsmb.3093 PMID: 26581521

12. Feng J, Funk W, Wang S, Weinrich S, Avilion A, Chiu C, et al. The RNA component of human telomerase. Science (80-). 1995; 269:1236–41. https://doi.org/10.1126/science.7544491 PMID: 7544491

13. Stohr BA, Blackburn EH. ATM mediates cytotoxicity of a mutant telomerase RNA in human cancer cells. Cancer Res. 2008; 68:5309–17. https://doi.org/10.1158/0008-5472.CAN-08-0504 PMID: 18593932

14. Marusíc L, Anton M, Tidy A, Wang P, Villepontea B, Bacchetti S. Reprogramming of telomerase by expression of mutant telomerase RNA template in human cells leads to altered telomeres that correlate with reduced cell viability. Mol Cell Biol. 1997; 17:6394–401. https://doi.org/10.1128/MCB.17.11.6394 PMID: 9343041

15. Sharaf R, Montesion M, Hopkins JF, Song J, Frampton GM, Albacker LA. A pan-cancer landscape of telomeric content shows that RAD21 and HGF alterations are associated with longer telomeres. Genome Med. 2022; 14:25. https://doi.org/10.1186/s13073-022-01029-7 PMID: 35227290

16. Varani G, McClain WH. The G-U wobble base pair. EMBO Rep. 2000; 1:18–23.

17. Crick FHC. Codon—anticodon pairing: The wobble hypothesis. J Mol Biol. 1966; 19:548–55. https://doi.org/10.1016/s0022-2836(66)80022-0 PMID: 5969078

18. Kim MM, Rivera MA, Botchkina IL, Shalaby R, Thor AD, Blackburn EH. A low threshold level of expression of mutant-template telomerase RNA inhibits human tumor cell proliferation. Proc Natl Acad Sci U S A. 2001; 98:7982–7. https://doi.org/10.1073/pnas.131211098 PMID: 11438744

19. Li S, Rosenberg JE, Donjacour AA, Botchkina IL, Hom YK, Cunha GR, et al. Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. Cancer Res. 2004; 64:4833–40. https://doi.org/10.1158/0008-5472.CAN-04-0953 PMID: 15256453

20. Cerone MA, Londôo-Vallejo JA, Autexier C. Mutated telomeres sensitize tumor cells to anticancer drugs independently of telomere shortening and mechanisms of telomere maintenance. Oncogene. 2006; 25:7411–20. https://doi.org/10.1038/sj.occ.1209727 PMID: 16767163

21. Gilley D, Lee MS, Blackburn EH. Altering specific telomerase RNA template residues affects active site function. Genes Dev. 1995; 9:2214–26. https://doi.org/10.1101/gad.9.18.2214 PMID: 7557376

22. Gilley D, Blackburn EH. Specific RNA residue interactions required for enzymatic functions of Tetrahymena telomerase. Mol Cell Biol. 1996; 16:66–75. https://doi.org/10.1128/MCB.16.1.66 PMID: 8524330
23. Lin J, Smith DL, Blackburn EH. Mutant telomere sequences lead to impaired chromosome separation and a unique checkpoint response. Mol Biol Cell. 2004; 15:1623–34. https://doi.org/10.1091/mbc.e03-10-0740 PMID: 14742705

24. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. Nature Publishing Group; 2013; 31:1023–31. https://doi.org/10.1038/nbt.2696 PMID: 24142049

25. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017; 9:34. https://doi.org/10.1186/s13073-017-0424-2 PMID: 28420421