Supplementary Material for

Highly Recurrent TERT Promoter Mutations in Human Melanoma

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/cgi/content/full/science.1229259/DC1)

Table S1 as an Excel file
**Materials and Methods:**

**Clinical samples.** All melanoma samples analyzed in this study were collected and sequenced under Institution-Review-Board-approved protocols.

**Identification and validation of TERT promoter mutations.** Whole-genome sequencing data corresponding to 25 melanoma tumor-normal pairs were interrogated for somatic mutations highly recurrent at the nucleotide level. 6 of 25 discovery pairs did not have adequate locus coverage, reducing the discovery set to 19 pairs. Polymerase chain reaction (PCR) was performed on genomic DNA followed by direct sequencing on amplified PCR products using an ABI 3730 DNA Sequence Analyzer on a subset of these tumor-normal pairs to verify the individual TERT promoter mutations as well as on an additional 51 pairs for further confirmation. Additionally the mutations were subcloned directly from tumor DNA using the TOPO (Invitrogen) cloning kit according to manufacturer’s instructions followed by Sanger sequencing (Fig. S1B). Oligonucleotide primers were synthesized according to the hg19 genomic reference sequence of TERT (genome.ucsc.edu). Primer sequences are available upon request.

The 70 tested melanomas represented 28 metastatic tumor samples, 11 primary tumor samples, and 31 tumor-derived cultures. Massively parallel sequencing was performed on cell lines as described previously and the TERT promoter was interrogated for recurrent mutations at coordinates chr 5:1,295,228 and chr 5:1,295,250 using MuTect. Chromatograms were viewed and screenshots taken with the Geneious software program (www.geneious.com). P-values and confidence intervals were calculated using standard packages in R (www.r-project.org).

**Plasmids.** Using normal germline DNA, a portion of the TERT core promoter (-132 to +5) or the full core promoter (-200 to +73) were cloned into the multiple cloning site of pGL3-Basic (Promega) upstream of the firefly luciferase gene with primers flanked with Mlu1 or Xho1 sites to create pGL3-TERT132wt, a wild-type TERT-luc promoter construct. Using this strategy, the C228T mutation was also cloned directly from tumor DNA into pGL3-Basic. Site-directed mutagenesis using QuikChange Lightning (Agilent) was performed on the wild-type promoter construct to produce the promoter
mutations. Constructs were verified by Sanger sequencing. All primer sequences are available upon request.

**Reporter assays.** A375 melanoma cells, RPMI 7951 melanoma cells, UACC-62 melanoma cells, T24 bladder cancer cells, and HepG2 hepatocellular carcinoma cells were seeded at a density of $3 \times 10^5$ cells in a 6-well format. T24 cells were cultured in McCoy’s 5A Medium (Life Technologies), HepG2 cells were cultured in Dulbecco’s Modified Eagle Medium (Life Technologies), A375 cells were cultured in Minimal Essential Medium (Life Technologies), RPMI cells and UACC-62 cells were cultured in RPMI media, all containing 10% fetal bovine serum. Cells were transfected the following day using Fugene 6 (Promega) with $2.25 \mu g$ of the $TERT$–luc promoter construct and $0.25 \mu g$ of pRL-TK (Promega), a control Renilla luciferase vector (12, 13). 48 hours later cells were lysed and luciferase activity was assayed with the Dual Luciferase Reporter (Promega) assay in a 96-well format according to manufacturer instructions. Experiments were performed in triplicate wells. Relative luciferase activity was calculated as the ratio of firefly to Renilla luciferase activity, to control for transfection efficiency. Control is the relative luciferase activity of cells transfected with promoterless reporter alone (pGL3-Basic).

**Figure S1. Identification of $TERT$ promoter mutations in melanoma**

(A) Representative screenshot of $TERT$ promoter mutations chr 5: 1,295,228 C>T (C228T) and chr 5: 1,295,250 C>T (C250T) from Integrative Genomics Viewer. Average depth of coverage in the 19 melanoma tumor-normal pairs with whole genome sequence coverage at the relevant loci was 58x in the tumor and 30x in the normal at chr 5: 1,295,228 and 61x in the tumor and 30x in the normal at chr 5: 1,295,250, with minimum base quality score of 30 and minimum read mapping quality of 60.

(B) Additional sequence chromatograms of matched tumor and normal DNA representing somatic mutations C228T and C250T in the $TERT$ promoter locus.

(C) Subcloning of $TERT$ core promoter mutations C228T and C250T. Sequence chromatograms depict the reverse complement G>A transition.
(D) Luciferase reporter assays for transcriptional activity from a portion of the TERT core promoter (-132 to +5) with either the C228T or C250T mutation compared to wild-type promoter in A375, T24 or HepG2 cell lines. The results depicted are the average of at least 3 independent experiments. Values are mean ± s.d. * P < 0.01.

Table S1. Recurrent TERT promoter mutation status of melanomas and CCLE cell lines

Melanoma samples and cell lines are listed with indicated TERT promoter mutation. Two samples had a dinucleotide mutation CC>TT causing C228T and C229T, were counted as C228T samples, and also generated a consensus ETS motif.

Supplementary References:


References and Notes


2. Materials and methods are available as supplementary materials on Science Online.


