Highly Recurrent TERT Promoter Mutations in Human Melanoma

Franklin W. Huang *et al.*

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1980 cohorts, and age is not a significant determinant within pre- and post-OCP periods. The results for neuroticism are less robust to checks for age effects (table S16).

Previous research has shown that noncognitive attributes such as conscientiousness, neuroticism, and optimism are important determinants of educational attainment, labor market outcomes, health, and marriage and divorce (*38*–*40*). Prosocial behavior is consistently seen to be an important determinant of social capital and plays a role in institutional development (*41*). A willingness to take risks is an important component of entrepreneurship (*17*). Our data show that being an only child as a result of the OCP is associated with taking less risk in the labor market (table S19).

Although our findings were obtained from a comparison of cohorts in Beijing born directly around the time of the policy's introduction, our results are generalizable to other urban areas of China where the OCP was strictly implemented. Previous work suggests that differences between only children and others in Beijing are similar to those in other urban areas (*26*). The effect of the policy on the behavior of people born long after the policy's introduction may, however, differ from what we found here, because later cohorts will have grown up with very limited extended family and in a society dominated by only children. Under such circumstances, we would expect that the policy's effect would, if anything, be magnified.

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Supplementary Materials

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Highly Recurrent *TERT* Promoter Mutations in Human Melanoma

Franklin W. Huang,[1,2,3]* Eran Hodis,[1,3,4]* Mary Jue Xu,[1,3,4] Gregory V. Kryukov,[1] Lynda Chin,[5,6] Levi A. Garraway[1,2,3]†

Systematic sequencing of human cancer genomes has identified many recurrent mutations in the protein-coding regions of genes but rarely in gene regulatory regions. Here, we describe two independent mutations within the core promoter of *telomerase reverse transcriptase* (*TERT*), the gene coding for the catalytic subunit of telomerase, which collectively occur in 50 of 70 (71%) melanomas examined. These mutations generate de novo consensus binding motifs for E-twenty-six (ETS) transcription factors, and in reporter assays, the mutations increased transcriptional activity from the *TERT* promoter by two- to fourfold. Examination of 150 cancer cell lines derived from diverse tumor types revealed the same mutations in 24 cases (16%), with preliminary evidence of elevated frequency in bladder and hepatocellular cancer cells. Thus, somatic mutations in regulatory regions of the genome may represent an important tumorigenic mechanism.

Systematic characterization of human cancer genomes has led to the discovery of a wide range of mutated genes that contribute to tumor development and progression. Most of the somatic mutations in tumors reside within the protein-coding regions of genes or at splice junctions. To determine whether tumor genomes harbor recurrent mutations outside of protein-coding regions, we systematically queried noncoding somatic mutations using published whole-genome sequencing data.

Analysis of whole-genome sequencing data from malignant melanomas (*1*, *2*) revealed two somatic *telomerase reverse transcriptase* (*TERT*) gene promoter mutations in 17 of 19 (89%) cases examined. The average sequence coverage at the *TERT* promoter locus was 30-fold in normal samples and 60-fold in tumor samples (fig. S1A).

[1]Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA. [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA. [3]Harvard Medical School, Boston, MA 02115, USA. [4]Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA. [5]Department of Genomic Medicine, M.D. Anderson Cancer Center, Houston, TX 77030, USA. [6]Institute for Applied Cancer Science, M.D. Anderson Cancer Center, Houston, TX 77030, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: levi_garraway@dfci.harvard.edu

Each of these promoter mutations resulted in a cytidine-to-thymidine transition at a dipyrimidine motif indicative of ultraviolet (UV) light–induced damage (chr5, 1,295,228 C>T and 1,295,250 C>T; hereafter termed C228T and C250T, respectively), and both mutations localized within 100 base pairs (bp) of the *TERT* transcriptional start site (TSS) (mean allelic fraction, 0.32; range, 0.07 to 0.55) (table S1). We validated these mutations by means of polymerase chain reaction and Sanger sequencing tumor/normal sample pairs from both the discovery set (Fig. 1A and fig. S1, B and C) and an extension set of 51 additional melanoma tumor/normal sample pairs. Within this extension set, 33 tumors (65%) harbored one of the mutations. Moreover, the mutations were mutually exclusive in both the discovery and extension sets ($P = 5.4 \times 10^{-7}$, Fisher's one-sided exact test). Two tumors with a C228T transition also contained an adjacent C>T transition (at position chr5, 1,295,229), which is indicative of a dinucleotide CC>TT transition. Together, these *TERT* promoter mutations were observed in 50 of 70 (71%; 95% confidence interval: 59 to 82%, Clopper-Pearson method) melanomas examined (Fig. 1B and table S1).

Both C228T and C250T generated an identical 11-bp nucleotide stretch (5′-CCCCTTCCGGG-3′) containing a consensus binding site for E-twenty-six (ETS) transcription factors (GGAA, reverse complement) within the *TERT* promoter region. Because ETS transcription factors may become activated through dysregulation of mitogen-activated protein kinase (MAP kinase) signaling, we hypothesized that these promoter mutations might augment gene expression. To test this hypothesis, we used a reporter assay system in which the relevant portion of the mutant or wild-type *TERT* core promoter was cloned upstream of the firefly luciferase gene (*2*). Here, we tested both a core promoter fragment (–132 to +5 relative to the TSS) and the full core promoter (–200 to +73). In comparison to the wild-type *TERT* promoter, both mutations conferred approximately two- to fourfold increased transcriptional activity in five distinct cell line contexts (Fig. 1C and fig. S1D). Thus, each mutation was capable of augmenting transcriptional activity from the *TERT* promoter.

To investigate whether similar *TERT* promoter mutations occur in other cancer types, we examined sequencing data from this locus in 150 cell lines from the Cancer Cell Line Encyclopedia (CCLE) (*3*). Overall, 24 CCLE lines (16%) contained either C228T or C250T (mean allelic fraction, 0.61; range, 0.17 to 1.00) (table S1). An increased frequency in melanoma was again noted (five of six lines tested), with additional evidence suggesting possible heightened prevalence (>25%; one-sided 95% confidence interval) in bladder (three of three lines) and hepatocellular cancer cell lines (four of six lines) (Fig. 1D).

Several lines of evidence support the hypothesis that these promoter mutations may function as driver events that contribute to oncogenesis through TERT dysregulation and undergo positive selection, at least in human melanoma. First, the *TERT* promoter mutations showed a combined frequency that exceeded those of *BRAF* and *NRAS* mutations, which activate known melanoma driver oncogenes (*4*, *5*). In an analysis restricted to somatic mutations present at an allelic fraction of 0.2 or greater [to reduce artifacts of mutation calling (*1*)], the four most recurrent melanoma nucleotide substitutions included *BRAF* [chr7, 140,453,136 A>T (V600E)], *NRAS* [chr1, 115,256,529 T>C (Q61R)], and the *TERT* core promoter mutations C228T and C250T. Second, although highly recurrent, C228T and C250T occurred in a wholly mutually exclusive fashion. This suggests the possibility that the mutations might be functionally redundant. Third, the absence of other recurrent somatic mutations in the 3 kb upstream of the *TERT* transcription start site in the queried melanomas (*1*) coupled with the absence of the described *TERT* promoter mutations in 24 lung adenocarcinomas with comparably high somatic mutation rates (*6*) reduces the possibility that these recurrent *TERT* promoter mutations are solely due to an increased background mutation rate at this locus.

Although the role of telomerase in tumorigenesis is well established, details regarding its dysregulation in cancer cells remain incompletely understood, particularly in melanoma (*7*). The *TERT* promoter mutations identified here may link telomerase gene regulation and tumorigenic activation in this malignancy. The high prevalence of C228T and C250T suggests that these *TERT* promoter mutations may comprise early genetic events in the genesis of melanoma and other cancer types. Although TERT expression alone is not sufficient to bypass oncogene-induced senescence, genomic *TERT* activation may potentiate mechanisms by which melanocytes achieve immortalization in the setting of oncogenic mutations (*8*). These results therefore suggest that renewed efforts to develop clinically effective telomerase inhibitors may be warranted.

At the same time, promoter mutations likely represent only one potential mechanism of *TERT* reactivation in a subset of human cancers. Indeed, recurrent chromosomal copy gains spanning the *TERT* locus have been described previously for several cancers, including melanoma (*9*, *10*).

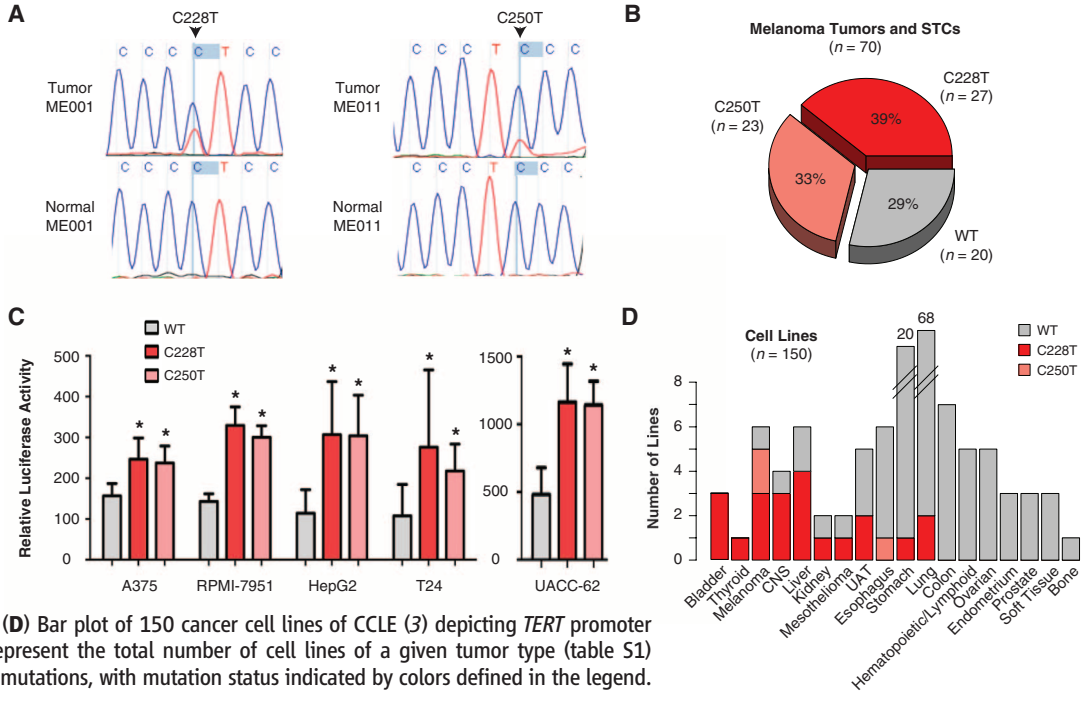

Fig. 1. Identification of *TERT* promoter mutations in melanoma and cancer cell lines. (**A**) Sequence chromatograms of matched tumor and normal DNA representing somatic mutations chr 5 [1,295,228 C>T (C228T)] and chr 5 [1,295,250 C>T (C250T)] in the *TERT* promoter locus. (**B**) Pie chart of C228T and C250T somatic mutation status in 70 surveyed melanoma tumors and short-term cultures. Sum of percentages is greater than 100% because of rounding. (**C**) Luciferase reporter assays for transcriptional activity from the *TERT* core promoter (–200 to +73) with either the C228T or C250T mutation compared with wild-type promoter in A375, RPMI-7951, UACC-62, T24, or HepG2 cell lines. The results depicted are the average of at least three independent experiments. Values are mean ± SD; *$P < 0.05$. (**D**) Bar plot of 150 cancer cell lines of CCLE (*3*) depicting *TERT* promoter mutation status. Individual bars represent the total number of cell lines of a given tumor type (table S1) interrogated for C228T and C250T mutations, with mutation status indicated by colors defined in the legend.

Highly recurrent somatic mutations within a cancer gene promoter region have not previously been described. Similarly, the de novo mutational generation of transcription factor binding motifs in tumor genomes was heretofore unknown, although an ETS transcription factor binding motif was previously associated with a single-nucleotide polymorphism insertion at the MMP-1 locus (*11*). Together, these findings raise the possibility that recurrent somatic mutations involving regulatory regions, in addition to coding sequences, may represent important driver events in cancer.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1229259/DC1
Materials and Methods
Fig. S1
Table S1
References (*12*, *13*)

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TERT Promoter Mutations in Familial and Sporadic Melanoma

Susanne Horn,[1,2] Adina Figl,[1,2] P. Sivaramakrishna Rachakonda,[1] Christine Fischer,[3] Antje Sucker,[2] Andreas Gast,[1,2] Stephanie Kadel,[1,2] Iris Moll,[2] Eduardo Nagore,[4] Kari Hemminki,[1,5] Dirk Schadendorf,[2]*† Rajiv Kumar[1]*†

Cutaneous melanoma occurs in both familial and sporadic forms. We investigated a melanoma-prone family through linkage analysis and high-throughput sequencing and identified a disease-segregating germline mutation in the promoter of the *telomerase reverse transcriptase* (*TERT*) gene, which encodes the catalytic subunit of telomerase. The mutation creates a new binding motif for Ets transcription factors and ternary complex factors (TCFs) near the transcription start and, in reporter gene assays, caused up to twofold increase in transcription. We then screened the *TERT* promoter in sporadic melanoma and observed recurrent ultraviolet signature somatic mutations in 125 of 168 (74%) of human cell lines derived from metastatic melanomas, 45 of 53 corresponding metastatic tumor tissues (85%), and 25 of 77 (33%) primary melanomas. The majority of those mutations occurred at two positions in the *TERT* promoter and also generated binding motifs for Ets/TCF transcription factors.

The identification of germline mutations that cosegregate with disease in cancer-prone families often provides genetic and mechanistic insights into the more common, sporadically arising cancers. In a study of cutaneous melanoma, the most malignant skin cancer, we investigated a large pedigree with 14 related melanoma patients who were not carriers of germline mutations in *CDKN2A* or *CDK4*, two known melanoma genes (Fig. 1). Multipoint linkage analysis showed a possible 2.2-Mb linkage region on chromosome 5p with maximal logarithm of the odds ratio for linkage scores of 2.35 at rs1379917 and 2.45 at rs1968011. Target-enriched high-throughput sequencing (HTS) of the region was carried out on constitutional DNA from the four affected and four unaffected members of the family with an average coverage between 55- and 108-fold (table S1) (*1*). The HTS data revealed a single promoter variant, three intronic variants, and three nongene variants previously unknown and unique to the DNA sequences of the affected individuals (table S2). The disease segregating variants, seven in total, were validated by Sanger sequencing of DNA from the individuals sequenced by HTS and of DNA from additional unaffected members of the family. The new variants were also detected in an unaffected member (754, table S3), who was 36 years old and carried multiple nevi. DNA from affected individuals other than those sequenced by HTS was not available for testing.

Of the seven unique variants identified, one variant (T>G), was located in the promoter at –57 base pairs (bp) from ATG translation start site of the *telomerase reverse transcriptase* (*TERT*) gene. The *TERT* gene encodes the catalytic reverse transcriptase subunit of telomerase, the ribonucleoprotein complex that maintains telomere length. The nucleotide change in the sequence CCTGAA>CCGGAA creates a new binding motif for Ets transcription factors, with a general recognition motif GGA(A/T). Beyond the general motif for Ets transcription factors, the familial mutation also generates a binding motif, CCGGAA, for the ternary complex factors (TCFs) Elk1 and Elk4 (*2*, *3*). To exclude the possibility that the detected promoter mutation in *TERT* is a common germline variant, we screened germline DNA from 140 sporadic melanoma cases and 165 healthy controls, and none carried the variant. Screening of DNA from index cases from 34 Spanish melanoma families also did not show any mutations. No carriers were found in dbSNP and the 1000 Genomes databases (data available for 18 individuals were obtained from Ensembl).

The familial mutation in the *TERT* promoter was in complete allelic linkage with a common polymorphism rs2853669 (G>A) at –246 bp upstream from the ATG start site (table S3). In previous work, this polymorphism was reported to disrupt an Ets binding site, and it was associated with low telomerase activity in patients with non–small cell lung cancer (*4*). In luciferase reporter gene assays, we found that the activity of constructs containing the mutation at –57 bp of the *TERT* promoter was increased 1.5-fold and 1.2-fold over the wild-type construct in Ma-Mel-86a and human embryonic kidney (HEK) 293T cells, respectively. A construct with both the *TERT* mutation and the variant allele of the rs2853669 polymorphism showed a 2.2-fold increase in promoter activity in Ma-Mel-86a and and 1.3-fold increase in HEK293 cells (mean from three measurements; details in supplementary text and fig. S1).

The germline occurrence of the promoter mutation, creating an Ets/TCF motif, can result in modification of *TERT* expression in all tissues expressing Ets/TCF. Highest staining for the TCF Elk1 protein has been reported in female-specific tissues, such as ovary and placenta. The increased expression of TCF Elk1 protein in female-specific tissues may cause gender-related differences in

[1]Division of Molecular Genetic Epidemiology, German Cancer Research Center, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany. [2]Department of Dermatology, University Hospital Essen, 45122 Essen, Germany. [3]Institute of Human Genetics, University of Heidelberg, 69120 Heidelberg, Germany. [4]Department of Dermatology, Instituto Valenciano de Oncologia, Valencia, Spain. [5]Center for Primary Health Care Research, Lund University, Malmö, Sweden.

*To whom correspondence should be addressed. E-mail: r.kumar@dkfz.de (R.K.); dirk.schadendorf@uk-essen.de (D.S.)
†These authors contributed equally to this work.