TERT promoter mutations and monoallelic activation of TERT in cancer

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SHORT COMMUNICATION

TERT promoter mutations and monoallelic activation of TERT in cancer

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Here we report that promoter mutations in telomerase (TERT), the most common noncoding mutations in cancer, give rise to monoallelic expression of TERT. Through deep RNA sequencing, we find that TERT activation in human cancer cell lines can occur in either mono- or biallelic manner. Without exception, hotspot TERT promoter mutations lead to the re-expression of only one allele, accounting for approximately half of the observed cases of monoallelic TERT expression. Furthermore, we show that monoallelic TERT expression is highly prevalent in certain tumor types and widespread across a broad spectrum of cancers. Taken together, these observations provide insights into the mechanisms of TERT activation and the ramifications of noncoding mutations in cancer.

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INTRODUCTION

In healthy tissues, expression of the telomerase gene (TERT) is generally restricted to the germline and stem cells. However, TERT activation, necessary for the maintenance of unlimited replicative potential, occurs commonly in many cancers. Recurrent mutations in the TERT promoter region were first discovered in melanoma and have since been found to be the most common somatic mutations in many cancers.1-4 Two mutually exclusive cytidine-to-thymidine mutations, C228T and C250T (chr5:1 295 228 C>T and 1 295 250 C>T; hg19), positioned at 124 and 146 base pairs upstream of the ATG translational start site of TERT, respectively, are found in ~70% of melanomas, 80-90% of glioblastoma multiforme, 60% of hepatocellular carcinoma, 60% of bladder cancer, 70% of basal cell carcinoma, 50% of cutaneous squamous cell carcinoma and up to 30% of thyroid cancers. Other recurrent but much less frequent mutations in the TERT promoter (occurring at positions chr5: 1 295 191, 1 295 228, 1 295 242-1 295 243 CC>TT and 1 295 161 A>C) have also been observed.5 All of these mutations create putative consensus ETS transcription factor binding sites (GGAA/T) and are hypothesized as a mechanism of TERT activation.6-7 Subsequent studies in glioblastoma and bladder cancer demonstrated that TERT promoter mutations correlate with higher TERT mRNA and protein expression and elevated telomerase activity.6 Furthermore, recent data demonstrate that the mutant TERT promoter can be bound and activated by GABP, an ETS transcription factor.7 The recurrent TERT promoter mutations also prevent silencing of TERT upon differentiation of stem cells.8 For this study, we used whole-genome DNA and deep RNA sequencing data from 329 cancer cell lines representing a wide range of tumor types from the Cancer Cell Line Encyclopedia (CCLE)9,10 to interrogate the TERT locus and determine the modes of TERT activation.

RESULTS AND DISCUSSION

To analyze TERT expression, we first identified cell lines with heterozygous ‘anchor’ single-nucleotide polymorphisms (SNPs) (defined here as having at least three reference and alternate reads with a DNA alternate allelic fraction between 0.25 and 0.75) in the DNA sequences of expressed exonic and untranslated regions of the TERT gene. Cell lines with insufficient RNA sequencing coverage of anchor SNPs (i.e., fewer than eight reads) and cell lines with high-level copy-number amplification or deletion of the TERT locus were excluded from downstream analyses. In total, 88 CCLE lines met these criteria and had at least one heterozygous SNP that would allow evaluation of an allelic bias in RNA expression (Supplementary Table 1). The number of sequencing reads that supported the reference or alternative alleles were counted both in whole-genome sequencing and RNA-Seq data and used to quantify allelic imbalance (Figure 1a). In total, 39 out of 88 cell lines (44.3%) showed a monoallelic pattern of TERT expression with a cutoff of one allele being expressed at least 10-fold higher than the other allele in RNA versus DNA.

We then investigated the relationship between the monoallelic expression pattern and known mechanisms of TERT activation. To identify the recurrent TERT promoter mutations, we used whole-genome sequencing data from the CCLE to interrogate the TERT promoter region in 329 cell lines, of which 316 lines had sufficient coverage at all previously defined hotspots (Supplementary Table 2 and Supplementary Figure 1). Promoter mutation status was determined by computing allele counts at genomic loci for five known recurrent somatic mutations in the TERT gene promoter region (positions 1 295 191, 1 295 228, 1 295 242, 1 295 243 and 1 295 250 in chromosome 5; Supplementary Table 2). Samples in which mutations were detected at any of these positions were classified as having one or both alleles mutated in the TERT promoter.
TERT almost exclusively showed monoallelic expression of pancreas, upper aerodigestive tract, urinary tract and stomach Cancer types such as melanoma, multiple myeloma, TERT varied markedly among different cell lineages (Figure 2). Overall, in over 70% of lineages, at least one cell line displayed monoallelic expression or biallelic expression of differences in mRNA expression levels between cell lines that in other models (Supplementary Figure 3). Phenomenon that has been observed in monoallelic expression TERT showed evidence of monoallelic activation of Overall, in over 70% of lineages, at least one cell line displayed monoallelic expression or biallelic expression of these data suggest that somatic monoallelic activation of TERT may show evidence of a transcriptional compensation phenomenon that has been observed in monoallelic expression in other models. To test whether the promoter mutations occurred in cis with the alleles, which showed monoallelic expression, we subclassed the region of the TERT gene encompassing the heterozygous ‘anchor’ SNP (rs2736098) at position chr5:1 294 086 and the promoter mutation C228T (chr5:1 295 228 C->T) in four unrelated cell lines.

Figure 1. Identification of cancer cell lines with monoallelic TERT expression. (a) Fraction of sequencing reads harboring nucleotide that differs from reference human genome for heterozygous anchor SNPs. DNA and RNA alternate allelic fractions are plotted along the x axis and y axis, respectively. Analysis was performed on 88 cell lines with a DNA alternate allelic fraction between 0.25 and 0.75 in the DNA sequences of expressed exonic and untranslated regions of the TERT gene. Strong deviation from the diagonal indicates allelic bias in expression. Individual points represent samples, and those harboring hotspot promoter mutations C228T, C228A or C250T and were found within the 88 cell lines that met the criteria for assessment of allelic bias. (b) Bar plot of promoter mutation status in cell lines with monoallelic and biallelic expression of the TERT gene. TERT expression was classified as monoallelic in samples with heterozygous anchor SNPs in the TERT gene for which expression of a major allele was >10-fold higher than expression of the minor allele in RNA versus DNA.

Figure 2. Distribution of cell lines with monoallelic TERT expression across tissue lineages. We found a highly significant difference in the distribution of samples with monoallelic TERT expression across lineages when compared with cell lines with biallelic TERT expression for both promoter-mutant and promoter-wild-type cell lines (P = 1.59 × 10^−6 and P = 0.006, respectively; Fisher–Freeman–Halton test).

Using Sanger sequencing, we found that the allele that showed monoallelic expression at this position was in cis with the mutant promoter allele and not the wild-type promoter allele in all lines tested (P = 0.05; Student’s T-test; Figure 3).

Although all cell lines with TERT promoter mutations showed a monoallelic pattern of TERT expression, these cell lines accounted for only half of the samples that exhibited strong allelic bias. Monoallelic expression in the remaining samples could be potentially explained by two major mechanisms—unidentified cis-acting genetic alterations that affect only one chromosome, or mitotically stable epigenetic activation of one allele. To investigate the first possibility, we analyzed the 10 kb region upstream of the
account for the non-promoter-mutant-associated TERT monoallelic expression remains an active area of investigation.15–17 Aside from TERT, recent studies demonstrate that other promoter regions may be recurrently mutated in cancer.18–20 Our findings suggest that somatic monoallelic activation of TERT is a common mechanism of TERT expression and that TERT promoter mutations drive monoallelic expression of TERT.

CONFLICT OF INTEREST
Drs Sellers and Stegmeier are employees of Novartis. Dr Garraway has received research funding form Novartis and holds equity in Foundation Medicine. The other authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Oncogenesis website (http://www.nature.com/oncsis).