TERT gene: its function and dysregulation in cancer

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ABSTRACT

In this review, we summarise the function and structure of telomerase reverse transcriptase (*TERT*) in humans, including its regulation. The dysregulation of telomerase through *TERT* promoter mutations across a range of cancers is discussed. The molecular mechanism activated by *TERT* promoter mutations is outlined. Finally, the timing of *TERT* promoter mutations during carcinogenesis is reviewed in the context of their potential utility as clinical biomarkers of malignant transformation.

INTRODUCTION

The immortalisation of cancer cells is a critical hallmark required for malignant behaviour.[1] The reactivation of telomerase by tumours is the most common pathway contributing to the immortalised state, and in turn the most common somatic event to achieve this involves mutations in the promoter of telomerase reverse transcriptase (*TERT*), the catalytic subunit of telomerase. By contrast, deleterious germline *TERT* mutations have been shown to be associated with *dyskeratosis congenita* (a progeria) and pulmonary fibrosis. As a result, there is interest in understanding *TERT*, its regulation and dysregulation. More specifically, elucidating the molecular mechanism of how promoter mutations in *TERT* lead to increased expression of telomerase during carcinogenesis and how these alterations could potentially be exploited for diagnostic and therapeutic purposes is of great interest to researchers and clinicians.

The structure and function of *TERT*

The linear organisation of eukaryotic chromosomes results in two fundamental problems: first, the ends of the chromosomes being recognised as double-stranded breaks by the cell, and second the erosion of the 5' end during cycles of genomic replication due to non-reproduction of the RNA primer binding site.[2]

The former problem is avoided by the presence of telomeres at the ends of chromosomes. Telomeres are tandem repeats of TTAGGG (in vertebrates and most metazoans[3]) which, when assembled with a protein complex known as shelterin, protect the ends of chromosomes from DNA repair pathways initiated by a DNA damage response.

The latter problem, known as the 'end replication problem', can be rectified by telomerase. The ribonucleoprotein telomerase has a core component composed of a reverse transcriptase (TERT) subunit and a separately coded RNA template (TERC or TR), which, along with a series of associate proteins, leads to the extension and replenishment of telomeres.

In humans, telomerase is normally expressed during development in embryonic stem cells and later silenced in somatic cells on differentiation.[4] Telomerase expression is maintained to a restricted set of cells, primarily transit-amplifying stem-like cells and germ cells.

In the absence of telomere expansion, the proliferative capacity of human cells is limited by the continuous erosion of telomeres, leading to cellular senescence. The number of cellular divisions prior to senescence is known as the Hayflick limit.[5] This senescence response is a critical tumour suppressive mechanism, and yet can be the source of various diseases as the result of short telomeres, such as lung fibrosis.[6]

The role of *TERT* in cancer

Telomerase is detectable in over 90% of human cancers,[4 7] and its re-expression is thought to represent the most common pathway to cellular immortalisation. It has been shown that the increased expression of *TERT* leads to restoration of telomerase activity,[8 9] thereby implicating the transcriptional control of *TERT* as a key factor in diseases, including ageing and cancer.

During neoplastic progression, cellular senescence is bypassed by the inactivation of cell cycle checkpoint genes such as p53 and pRb.[10] With continuing cycles of cellular division past the point of replicative senescence (M1 stage), telomeres become critically shortened and cells enter 'crisis' (M2 stage). In crisis, chromosome end fusions and breakage-fusion-bridge events occur, leading to chromosomal instability. Cells escape crisis through either upregulation of telomerase or the alternate lengthening of telomeres mechanism.[11] In the latter process, telomeres are replenished using homologous recombination.

TERT is located on chromosome 5 and consists of 16 exons.[12 13] The protein product TERT exists as a dimer within the telomerase holoenzyme[14] and consists of three regions: (1) the N-terminal extension that contains an N-terminal domain and a telomerase RNA-binding domain; (2) the central catalytic reverse transcriptase domain; and (3) the C-terminal extension.[15] This basic organisation has remained remarkably stable over evolutionary time,[16] implying that it was present in basal metazoans at the emergence of multicellularity. Interestingly, *TERT* is not present in *Drosophila*, as this genus does not use telomerase to solve the end replication problem.[17]

The promoter region of *TERT* has been extensively studied in order to identify the candidate factors involved in influencing *TERT* expression. (For a comprehensive review of the promoter structure and binding partners, see ref 18.) The *TERT*

promoter is guanine-cytosine rich (GC-rich)and lacks both a TATA box and a CAAT box. Multiple factors have been shown to influence TERT expression in context-specific ways, either singly or in coordination, including Myc, oestrogen, SP1, nuclear factor kappa B (NF-κB), p53, activator protein 1 (AP-1) and E2F factors. Recognition sequences for binding of p53, p21, SP1, E26 transformation-specific (ETS), E2F, AP-1, hypoxia-inducible factor 1 (HIF1) and c-myc are present within the promoter region.[18] Interestingly, the ETS family member gene *GABPA*, which binds *TERT* promoter mutations in cancer (described below), does not appear to bind the native promoter.[19]

Splice variants of *TERT*

While the transcriptional regulation of *TERT* has been studied in depth, recent work has evaluated the role of alternate splicing of mRNA transcripts. *TERT* can be translated from multiple differently spliced transcripts, with only the longest variant having reverse transcriptase enzymatic activity.[20] Breast cancer cell lines with overexpression of transcripts without catalytic function have been shown to have reduced apoptosis, conferring a survival advantage.[21] This suggests novel functions of TERT beyond telomere extension.

TERT upregulation in cancer/*TERT* promoter mutations

TERT can be upregulated in cancer through several mechanisms, including gene amplification, rearrangements and mutations within its promoter. *TERT* has been shown to be amplified in approximately 4% of cancers, especially ovarian cancer, lung adenocarcinoma, lung squamous cell carcinoma, oesophageal carcinoma and adrenocortical carcinoma.[22] Rearrangements of the *TERT* promoter have been demonstrated in high-risk neuroblastomas.[22 23] However, amplifications and rearrangements of *TERT* appear to be relatively rare overall in cancer, although common in specific subtypes.[22]

The identification of frequent non-coding *TERT* promoter mutations in cutaneous melanoma[24 25] at once simultaneously delineated a novel class of cancer driver mutations, as well as indicated the mechanism by which *TERT* upregulation occurred in a large proportion of tumours. These mutations are recurrent C>T transitions occurring at chr5:1295228 (−124 or C228T) or chr5:1295250 (−146 or C250T) (hg19/GCRh37 genome coordinates) within the core promoter of *TERT*, leading to the creation of novel ETS transcription factor binding sites. Initial studies using a luciferase reporter assay demonstrated increased *TERT* gene expression as a result of these mutations.[24]

TERT promoter mutations were subsequently demonstrated across a range of malignancies. Of note, the frequency of *TERT* promoter mutations varies considerably with tumour subtype. In melanoma, up to 80% of non-acral cutaneous melanomas have been shown to have *TERT* promoter mutations,[26–29] while less than 10% of acral melanomas[30] and less than 1% of uveal melanomas[31] have these mutations. In tumours of the central nervous system, *TERT* promoter mutations are present in approximately 70% of primary glioblastomas and oligodendrogliomas, but are relatively infrequent in secondary glioblastomas.[32–35] *TERT* promoter mutations are also common in urothelial carcinoma,[36 37] poorly differentiated and anaplastic thyroid carcinomas,[38 39] cutaneous basal cell and squamous cell carcinomas,[40 41] and hepatocellular carcinoma[42] (comprehensively and recently reviewed in ref 43).

The mutations in C228T and C250T, which rarely co-occur,[26 27] both lead to the creation of an identical 11-base sequence which corresponds to a de novo binding site for an ETS transcription factor (figure 1). Reporter assays have demonstrated that both C228T and C250T lead to increased transcription of *TERT*,[24 25] approximately doubling wild-type promoter expression. Increased telomerase expression has also been demonstrated in urothelial carcinomas with *TERT* promoter

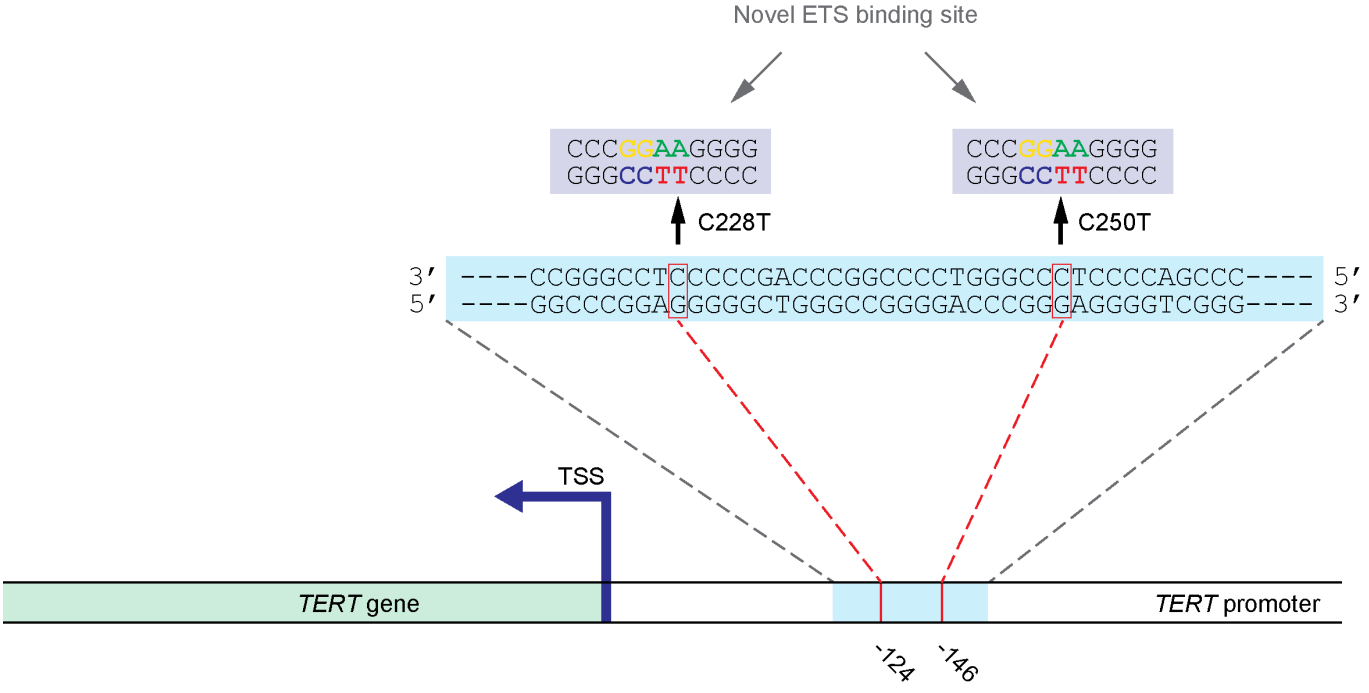

Figure 1 *TERT* promoter mutations occur most commonly at two positions. Two C→T mutations located at −124 and −146 base pairs from the TSS within the core promoter of *TERT* account for the majority of *TERT* promoter mutations identified in cancer. These mutations lead to the creation of novel ETS transcription factor binding sites which drive increased expression of *TERT*. ETS, E26 transformation-specific; TERT, telomerase reverse transcriptase; TSS, transcriptional start site.

mutations.[36] Additionally, *TERT* promoter mutations are associated with switching of inactive to active chromatin marks in the *TERT* promoter.[44] The likely ETS factor responsible for increased expression has been identified as GABPA,[45] which is the only one of the 26 members of the ETS family that forms multimeric complexes when driving gene expression. A recent study examining glioblastoma cell lines found GABPβ1L to be the critical binding partner of GABPA in driving *TERT* expression in the presence of a *TERT* promoter mutation.[19] Another group has suggested that the C228T and C250T mutations are functionally distinct: the former leading to GABPA recruitment; the latter leading to the generation of both an ETS site and a functional p52 site requiring ETS1/2 as the culpable ETS factor.[46]

The mechanism by which *TERT* promoter mutations ultimately facilitate cancer growth is still being elucidated. Whereas the initial studies used reporter assays to demonstrate increased *TERT* expression, the demonstrated increase in *TERT* expression appears to be relatively modest, and several studies have failed to find a significant association between *TERT* promoter status and *TERT* expression.[28 36 47] However, modest expression of *TERT* may still be sufficient for tumour cells to have a selective advantage.[48] Comparison between matched naevi and adjacent melanoma demonstrates increased *TERT* expression in the malignant component with a *TERT* promoter mutation.[49] A recent study by Chiba *et al*[50] demonstrated that, by contrast, telomere length was shorter in the melanomas than in the adjacent naevi.[50] In this latter study, the investigators propose that *TERT* promoter mutations act in two stages, with the first phase having moderately increased *TERT* expression yet at a level insufficient to prevent telomere shortening, resulting in shorter telomeres and delayed replicative senescence. In the second phase, there is telomere-driven genomic instability and upregulated telomerase activity.

A recent systematic pan-cancer analysis of more than 2500 tumour genomes found very few potential recurrent non-coding driver point mutations aside from those in *TERT*.[51] The pan-cancer analysis confirmed earlier results which did not find other non-coding mutations in cancer leading to changes in gene expression.[47] Rather than being an exemplar of a potential novel class of non-coding driver mutations, *TERT* promoter mutations appear to be unique, likely a result of the peculiar biology of telomerase and its regulation.

Timing of *TERT* promoter mutations and their use as a biomarker

While *TERT* promoter mutations demonstrate that the cells of origin of specific tumours have had downregulated *TERT* at some point, it is not clear at what point during tumourigenesis these mutations are acquired. Given that the selective pressure for *TERT* promoter mutations should be strongest near the Hayflick limit after multiple rounds of replication,[5] and therefore relatively late, it is somewhat unexpected that *TERT* promoter mutations are early events in carcinogenesis. Indeed, accumulating evidence suggests that *TERT* promoter mutations, at least in some tumours, are not an initiating event, but are selected for early in neoplasia, at an intermediate, preinvasive phenotype.[52–54] As such, *TERT* promoter mutations are potentially a useful diagnostic biomarker, as these lesions can potentially be treated with curative intent. However, in certain lesions, *TERT* promoter mutation detection will not distinguish diagnostically between malignant and intermediate entities.

The promise of the use of *TERT* promoter mutations as a clinical biomarker for cancer are threefold: first, that these mutations appear as a manifestation of a specific hallmark of cancer (although not fully tested yet in benign tissue)[1]; second, that these occur early in tumour development[49 52–55]; and third, that these mutations are recurrent in relatively few genomic positions (with the two canonical −124 and −146 point mutations accounting for over 85% of described mutations) in a range of malignancies.[43 55]

TERT promoter mutations have multiple potential uses as clinical biomarkers. *TERT* promoter mutations can be detected in urine samples from patients with recurrent urothelial carcinoma.[56] In melanoma, several approaches using droplet digital PCR have been recently developed[57 58] which can monitor *TERT* promoter mutations in serum. Moreover, *TERT* promoter mutation detection indicates a worse prognosis in thyroid malignancies,[59] melanoma[27] and gliomas.[60 61]

TERT in conditions other than cancer

Germline mutations in *TERT* have been associated with idiopathic pulmonary fibrosis,[62] and rare cases of germline mutations in *TERT* have been shown in families with *dyskeratosis congenita*.[63] *TERT* mutations have also been found in patients with severe emphysema.[64] Interestingly, somatic mutations in the *TERT* promoter have been found in the leucocytes of some individuals with germline mutations in *TERT*, suggesting that these mutations act as counterbalances to one another regarding *TERT* expression.[65]

CONCLUSION

The *TERT* gene plays important roles in normal biology, and perturbations of its regulation play a critical role in a variety of pathological states, especially neoplasia. In particular, TERT plays a central role in modulating telomerase activity in tumours, conferring the hallmark of immortality on neoplastic clones. A deeper understanding of this gene is pertinent given its potential usage as a biomarker and the future development of possible therapeutic avenues.

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