TERT biology and function in cancer: beyond immortalisation

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Abstract

Evasion of replicative senescence and proliferation without restriction, sometimes designated as immortalisation, is one of the hallmarks of cancer that may be attained through reactivation of telomerase in somatic cells. In contrast to most normal cells in which there is lack of telomerase activity, upregulation of *TERT* transcription/activity is detected in 80–90% of malignant tumours. In several types of cancer, there is a relationship between the presence of *TERT* promoter mutations, *TERT* mRNA expression and clinicopathological features, but the biological bridge between the occurrence of *TERT* promoter mutations and the aggressive/invasive features displayed by the tumours remains unidentified. We and others have associated the presence of *TERT* promoter mutations with metastatisation/survival in several types of cancer. In follicular cell-derived thyroid cancer, such mutations are associated with worse prognostic features (age of patients, tumour size and tumour stage) as well as with distant metastases, worse response to treatment and poorer survival. In this review, we analyse the data reported in several studies that imply *TERT* transcription reactivation/activity with cell proliferation, tumour invasion and metastatisation. A particular attention is given to the putative connections between *TERT* transcriptional reactivation and signalling pathways frequently altered in cancer, such as c-MYC, NF-κB and B-Catenin.

Key Words

- ▶ cancer
- ▶ telomerase
- ▶ thyroid
- ▶ TERT

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Introduction

In 1965, Hayflick described that human cells in culture had a finite lifetime *in vitro*, being able to divide 40–60 times before they would reach a gradual cessation of mitotic activity, a state now known as replicative senescence, and this limit was coined Hayflick limit (Hayflick 1965). In 1973, Olovnikov proposed that due to semi-conservative DNA replication, linear DNA of eukaryotic chromosomes would shorten with each round of DNA replication (Olovnikov 1973). Later, it was described that telomeres shortened 50–200 bp at each round of division, until a critical limit was achieved and replicative senescence

was reached (Szostak & Blackburn 1982, Blackburn 1991, Greider 1991).

Telomeres are cellular nucleoprotein complexes whose main function is to maintain chromosomal integrity and genome stability (Blackburn 2001). Telomeres are formed by hexameric repeats of a 5'-TTAGGG-3' sequence ending in a 3' single-stranded overhang, the G-strand overhang (Moyzis *et al.* 1988, Makarov *et al.* 1997, Wellinger & Sen 1997) that protects the genomic DNA from the continued erosion of telomeres during cell division.

Telomerase is a ribonucleoprotein polymerase with reverse transcriptase activity, capable of reversing the

continued erosion of telomeres by adding 5'-TTAGGG-3' tandem repeats at the end of the chromosomes (Liu *et al.* 2004). In somatic cells, telomerase remains inactive, but its activity can be found in germ cells and in stem cells in self-renewing tissues (Liu *et al.* 2004).

Human telomerase is composed of two main core subunits: telomerase reverse transcriptase (TERT) that constitutes the catalytic subunit and a RNA component (TERC) that provides a template for telomerase elongation (Greider & Blackburn 1989, Lendvay *et al.* 1996, Lingner & Cech 1996). The telomerase complex comprises several other molecules (Liu *et al.* 2004); of all its components, TERT and TERC are essential for the reconstitution of telomerase activity (Ishikawa 1997, Weinrich *et al.* 1997, Beattie *et al.* 1998). TERC is widely expressed in most cell types and even in telomerase-negative cells (Meyerson *et al.* 1997, Nakamura *et al.* 1997), whereas TERT expression is highly regulated, being absent or only present in low levels in somatic cells (Cong *et al.* 1999, Yi *et al.* 1999, Li *et al.* 2003). Lymphocytes are the only group of somatic cells that present telomerase activity and TERT expression. This activity is highly regulated during development, differentiation and activation of both T and B lymphocytes (Broccoli *et al.* 1995, Chiu *et al.* 1996, Morrison *et al.* 1996, Weng *et al.* 1998).

A positive correlation between TERT mRNA expression and telomerase activity has been observed, thus suggesting that telomerase is primarily regulated by TERT gene expression (Cong *et al.* 1999, Yi *et al.* 1999, Li *et al.* 2003).

Human TERT gene is located on chromosome 5p15.33 (Cong *et al.* 1999, Bryce *et al.* 2000), spanning 16 exons and 40 kb (Cukusic *et al.* 2008). The TERT promoter region is considered the most important regulatory element for telomerase expression (Cukusic *et al.* 2008). It spans 330 bp upstream of the translational start site and 37 bp of exon 2 (Cong *et al.* 1999, Cukusic *et al.* 2008), where the 181 bp fragment upstream of the transcriptional start site is the core functional promoter essential for transcriptional activation of TERT in cancer cells (Takakura *et al.* 1999). The promoter region is rich in CpG dinucleotides and SP1 sites, lacking both TATA and CAAT boxes (Takakura *et al.* 1999). This 5' regulatory region is known to be enriched with transcriptional-binding sites/consensus that interact with both negative and positive regulators of TERT (Renaud *et al.* 2005, Cukusic *et al.* 2008), indicating a high level of regulation by multiple factors (Cong *et al.* 1999) at transcriptional level (Cifuentes-Rojas & Shippen 2012).

One of the hallmarks of cancer is the evasion of replicative senescence and proliferation without

restriction, designated as immortalisation, and one of the ways to attain this hallmark is through reactivation of telomerase in somatic cells (Liu *et al.* 2004). Telomerase activity has been consistently detected in 80–90% of malignant tumours (Kim *et al.* 1994, Shay & Bacchetti 1997).

In cancer cells, in contrast to most normal cells, upregulation of TERT transcriptional activity has been described (Wright *et al.* 1996, Takakura *et al.* 1999). Differential upregulation of TERT activity has been described in cancer cells due to several processes, including transcriptional regulation, alternate RNA splicing and post-translational modifications such as protein phosphorylation (Cong *et al.* 2002, Cukusic *et al.* 2008).

In 2013, two groups reported the presence of mutations in the TERT promoter in melanoma (Horn *et al.* 2013, Huang *et al.* 2013). Shortly after, several groups reported the presence of recurrent somatic mutations in other types of cancer, namely in central nervous system, bladder, liver, thyroid, skin and others (Killela *et al.* 2013, Liu *et al.* 2013a, Nault *et al.* 2013, Scott *et al.* 2013, Vinagre *et al.* 2013, Allory *et al.* 2014, Hurst *et al.* 2014, Wu *et al.* 2014).

The TERT promoter mutations, commonly detected in the -124 (C228T) and -146 (C250T) residues (C>T or G>A in the reverse strand) (Horn *et al.* 2013, Huang *et al.* 2013), create an 11-base nucleotide stretch 5'-CCCCCTCCGGG-3', which contains a consensus binding site, GGAA (in reverse complement), for E-twenty six (ETS) family of transcription factors, providing a basis to the biological relevance of these mutations (Horn *et al.* 2013, Huang *et al.* 2013). Most ETS factors are ubiquitously expressed in normal human tissues and may be found to be overexpressed in tumours or cancer cell lines (Oikawa & Yamada 2003).

These recently described mutations represent one of the possible explanations for telomerase reactivation and activity in cancer cells, which can ultimately lead to cell immortalisation.

TERT promoter mutations in thyroid cancer

Thyroid cancer is the most common endocrine neoplasia (Hegedus 2004), whose incidence has been increasing, 6% per year, worldwide over the last few decades (La Vecchia *et al.* 2015). The thyroid tissue is a conditional renewal tissue, displaying a very low proliferative rate. Human thyroid cells are believed to divide up to five times in the adult life (Dumont *et al.* 1991). In the thyroid gland, there is no well-defined stem cell population that

might constitute a pool of cells responsible for retaining the capacity of tissue renewal. Some authors described so-called solid cell nests (SCNs) of the thyroid, which are embryonic remnants of the ultimobranchial body: these may represent the thyroid stem cell pool, as they express several stem cell markers, namely telomerase (Reis-Filho *et al.* 2003, Preto *et al.* 2004).

Thyroid cancer displays less frequent telomerase activation than other cancer types; telomerase activity has been found in 48% of papillary thyroid carcinomas (PTC), 71% of follicular thyroid carcinomas (FTC) and 78% of poorly differentiated and anaplastic thyroid carcinomas (PDTC and ATC, respectively) (Aogi *et al.* 1999, Saji *et al.* 1999, Hoang-Vu *et al.* 2002, Takano *et al.* 2007).

Recent studies have demonstrated that, so far, *TERT* promoter mutations were only detected in thyroid follicular cell-derived cancer, such as differentiated thyroid carcinoma (DTC), which includes the PTC and FTC and in PDTC and ATC. These mutations were virtually absent in sporadic benign lesions such as goitre, adenoma or thyroiditis (Landa *et al.* 2013, Liu *et al.* 2013a,b, Vinagre *et al.* 2013, Wang *et al.* 2014). *TERT* promoter mutations were found in 5–26% of the PTC (Landa *et al.* 2013, Liu *et al.* 2013a,b, 2014, Vinagre *et al.* 2013, Liu & Xing 2014, Melo *et al.* 2014, Xing *et al.* 2014, Muzza *et al.* 2015, Qasem *et al.* 2015, Crescenzi *et al.* 2016), 0–36 % of the FTC (Liu *et al.* 2013a,b, 2014, Vinagre *et al.* 2013, Liu & Xing 2014, Melo *et al.* 2014, Muzza *et al.* 2015, Qasem *et al.* 2015, Crescenzi *et al.* 2016), 21–52% of PDTC (Landa *et al.* 2013, 2016, Liu *et al.* 2013b, Vinagre *et al.* 2013, Melo *et al.* 2014, Qasem *et al.* 2015) and in 13–50% of ATC (Landa *et al.* 2013, 2016, Liu *et al.* 2013b, Vinagre *et al.* 2013, Melo *et al.* 2014).

We and others have associated the presence of *TERT* promoter mutations with metastatisation/survival in several types of cancer, namely in melanoma (Populo *et al.* 2014), gliomas (Heidenreich *et al.* 2015) and thyroid carcinomas (Melo *et al.* 2014). Specifically, in thyroid cancer, our group and others reported that these mutations are associated with worse prognostic features (age of patients, tumour size and tumour stage), as well as with distant metastases, worse response to treatment and poor survival (Landa *et al.* 2013, Liu & Xing 2014, Liu *et al.* 2013a,b, Melo *et al.* 2014, George *et al.* 2015, Qasem *et al.* 2015, Nasirden *et al.* 2016). *TERT* promoter mutations were also associated with shorter survival and/or short disease-free survival in melanomas (Griewank *et al.* 2014, Populo *et al.* 2014, Nagore *et al.* 2016) and glioblastomas (Mosrati *et al.* 2015, Simon *et al.* 2015, Batista *et al.* 2016, Yuan *et al.* 2016).

In a large cohort of patients with metastatic thyroid carcinoma, *TERT* promoter mutations were more frequently detected in radioiodine-refractory distant metastasis than in lymph node metastasis or primary tumours (Melo M, da Rocha AG, Batista R, Vinagre J, Martins MJ, Costa G, Ribeiro C, Carrilho F, Leite V, Lobo C, Cameselle-Teijeiro JM, Cavadas B, Pereira L, Sobrinho-Simões M & Soares P, unpublished observations). These results suggest that *TERT* promoter mutations appear to have a role in distant metastatisation in thyroid carcinomas (Melo M, da Rocha AG, Batista R, Vinagre J, Martins MJ, Costa G, Ribeiro C, Carrilho F, Leite V, Lobo C, Cameselle-Teijeiro JM, Cavadas B, Pereira L, Sobrinho-Simões M & Soares P, unpublished observations). Landa and coworkers recently reported that *TERT* promoter mutations were subclonal in the few PTCs that harbour them, whereas they are clonal and highly prevalent in the more aggressive forms of the disease, PDTCs and ATCs, which suggest clone selection during tumour evolution (Landa *et al.* 2016).

In a *Tinea capitis* radiation context, *TERT* promoter mutations were detected in 12% of carcinomas and in 21% of adenomas, being the most frequent, the –146C>T and the tandem mutations (Boaventura *et al.* 2016). These results point that *TERT* promoter mutations in this background present a different mutational signature (higher frequency of the –146C>T), and although it may be an early genetic event, they do not seem pivotal to the carcinogenic process in this setting; it has been demonstrated that the –146C>T confers a decreased transcriptional activity capacity when compared to the –124C>T mutation (Rachakonda *et al.* 2013). This event does not appear to drive carcinogenesis, at least *per se*, as *TERT* promoter mutations are not more prevalent in the carcinomas arising in irradiated setting than those in the sporadic well-differentiated carcinomas (Boaventura *et al.* 2016).

Several associations between the co-occurrence of *TERT* promoter mutations and other genetic alterations, such as *BRAF* and *RAS* mutations, have been reported in the literature. In melanoma, *TERT* promoter mutations are the most frequent somatic alteration and consistently co-occur with *BRAF*/*RAS* mutations (Horn *et al.* 2013, Griewank *et al.* 2014, Heidenreich *et al.* 2014, Populo *et al.* 2014, Macerola *et al.* 2015, Landa *et al.* 2016). In thyroid carcinomas, a positive association between co-occurrence of *TERT* promoter and *BRAF* mutations and a trend for co-occurrence with *RAS* mutations in primary tumours have been described (Landa *et al.* 2013, Liu *et al.* 2013a,b, 2014,

Vinagre *et al.* 2013, Xing *et al.* 2014, Muzza *et al.* 2015, Crescenzi *et al.* 2016).

One of the possibilities to explain the synergy between the co-occurrence between *TERT* promoter and *BRAF/RAS* mutations is the activation of the MAPK and/or PI3-Akt pathways that will culminate in the increased expression of ETS transcriptional factors, that can bind to the novel binding consensus sites created by the mutations and in this way upregulate *TERT* expression (Horn *et al.* 2013, Huang *et al.* 2013). Several studies reported evidence that confirm this possibility for cooperation between *TERT* promoter and *BRAF/RAS* mutations. Vallarelli and coworkers (Vallarelli *et al.* 2016) described that in melanoma, *TERT* promoter mutations provided a direct link between *TERT* expression and MAPK pathway activation due to *BRAF* or *NRAS* mutations via the transcription factor ETS1, that would bind to the mutated promoter leading to the re-expression of *TERT*. Also, in non-small-cell lung cancers (NSCLC), Liu and coworkers described that *KRAS* mutations increased *TERT* mRNA expression and telomerase activity by activating the RAS/MEK pathway, which contributes to the aggressive phenotype of NSCLC (Liu *et al.* 2016b).

Even though there is a positive association between the co-occurrence of *TERT* promoter and *BRAF/RAS* mutations, there is not a consensus in the literature about the combined prognostic value of these events. Some groups report that co-occurrence of *TERT* promoter and *BRAF* mutations are associated with worse prognosis factors in thyroid cancer (Allory *et al.* 2014, Liu *et al.* 2014, 2016a, Xing *et al.* 2014, Song *et al.* 2016), whereas other authors have not found this association in other series (Melo *et al.* 2014, 2015, George *et al.* 2015, Nasirden *et al.* 2016).

Although associations have been established in several cancers between the occurrence of *TERT* promoter mutations, *TERT* mRNA expression levels and clinicopathological features, the biological bridge between the presence of *TERT* promoter mutations and the aggressive/invasive features displayed by the tumours remains unidentified. There are two possible scenarios, not mutually exclusive, that may explain this finding. The first is that with the immortalisation, *TERT* promoter-mutated cells become more resistant to cell death during invasion, circulation and homing steps and are therefore more successful in the establishment of metastases. The second one is that *TERT* promoter mutations can give metastatic advantages to the cells by modulating/regulating (through *TERT*-regulated transcription programs) proteins and/or pathways important for the metastatic process (motility, invasion and MMPs).

Regulators and targets of *TERT* within major signalling pathways altered in cancer

In cancer cells *TERT* is transcriptionally reactivated; several signalling pathways have been implicated in this process, namely c-MYC (Greenberg *et al.* 1999), nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) (Yin *et al.* 2000) and B-Catenin (Zhang *et al.* 2012). The main interactions of *TERT* with these signalling pathways are summarised in Table 1.

c-MYC has been described as a regulator of *TERT* transcription program, and this hypothesis was suggested in 1999, when Greenberg and coworkers described *TERT* gene as a direct transcriptional target of c-MYC. c-MYC binds to the E-box (5'-CACGTG-3') in the *TERT* promoter region and activates *TERT* promoter (Greenberg *et al.* 1999).

c-MYC is able to induce telomerase activity through direct control of *TERT* transcription as this region has several binding sites/consensus for c-MYC (Wu *et al.* 1999). Both in normal human mammary epithelial cells (HMECs) and normal human diploid fibroblasts, the increased telomerase activity leads to extension of cell life span and proliferation (Wu *et al.* 1999). In a model of vascular injury, *TERT* was *de novo* activated in the intima of the injured arteries, involving activation of NF- κ B (Bu *et al.* 2010). Isolated intimal smooth muscle cell (SMC) stimulation with basic fibroblast growth factor or tumour necrosis factor alpha (TNF alpha) led to increased *TERT* activity; this effect was dependent on c-MYC activation as abrogation of c-MYC expression, by mutation of c-MYC-binding site or by c-MYC competitor, abrogated the transcriptional activity (Bu *et al.* 2010). Inhibition of NF- κ B also attenuated *TERT* transcriptional activity by the reduction of c-MYC expression (Bu *et al.* 2010).

It seems that *TERT* also regulates c-MYC stability in cancer. *TERT* is responsible for stabilisation of c-MYC levels in chromatin, contributing to either activation or repression of its target genes. *TERT* regulates c-MYC ubiquitination and proteosomal degradation, independent of its telomeric role, suggesting a feed-forward mechanism able to potentiate c-MYC-dependent oncogenesis (Koh *et al.* 2015).

NF- κ B is one of the most mentioned pathways altered in cancer that controls *TERT* transcriptional program. In 2000, the existence of a NF- κ B-binding site in the *TERT* promoter, specific for p50 and p65 was described (Yin *et al.* 2000). The fusion with a basal SV40 promoter activates transcription and enhances the activity of the native *TERT* promoter in mouse hepatoma cells when treated with a NF- κ B stimulator (Yin *et al.* 2000).

Table 1 Summary of multiple interactions of TERT with major signalling pathways, namely c-MYC, NF- κ B and Wnt/B-Catenin pathways.

TERT	Interaction effects	Cell effects	References
c-MYC	Induction of TERT transcriptional activity Induction of telomerase activity	Extension of cell life span Cell proliferation Replicative longevity of vascular cells Activation or repression of its target genes Potentiation of c-MYC-dependent oncogenesis	(Wu <i>et al.</i> 1999) (Bu <i>et al.</i> 2010)
	Stabilization of c-MYC levels on chromatin c-MYC ubiquitination and proteosomal degradation	Extension of cell life span Cell proliferation Replicative longevity of vascular cells Activation or repression of its target genes Potentiation of c-MYC-dependent oncogenesis	(Koh <i>et al.</i> 2015)
NF- κ B	Induction of TERT transcription activity Induction of telomerase activity Repression of ROS-dependent activation Modulation of TERT nuclear translocation Recruitment of IL6 and TNF alpha Upregulation of MMPs 1, 3, 9 and 10	Repression of ROS-dependent intracellular signalling and gene induction Inflammation Cancer progression Invasion	(Yin <i>et al.</i> 2000) (Zuo <i>et al.</i> 2011) (Mattiussi <i>et al.</i> 2012) (Akiyama <i>et al.</i> 2003) (Ghosh <i>et al.</i> 2012) (Ding <i>et al.</i> 2013)
Wnt/B-Catenin	Induction of TERT transcription activity Induction of telomerase activity Activation of Wnt-dependent reporters	Stem cell pluripotency Cell proliferation Cancer progression	(Zhang <i>et al.</i> 2012) (Park <i>et al.</i> 2009)

In MM.1S cells, NF- κ B appears to have an important role in regulating telomerase activity by modulating nuclear translocation of *TERT* protein bound to NF- κ B, in response to TNF alpha stimulation (Akiyama *et al.* 2003). Also, in response to TNF alpha, it seems that human telomerase represses reactive oxygen species (ROS)-dependent intracellular signalling and gene induction (Mattiussi *et al.* 2012). In HepG2 cells, NF- κ B appears to modulate *TERT* mRNA levels in a dose-dependent manner by stimulation of NF- κ B pathway (p65) (Zuo *et al.* 2011). A feed-forward regulation between *TERT* and NF- κ B was suggested as telomerase directly regulates NF- κ B-dependent gene expression by binding to the NF- κ B p65 subunit (Ghosh *et al.* 2012). This leads to the recruitment of a subset of NF- κ B promoters such as Interleukin 6 (IL6) and TNF alpha. These cytokines, which are critical for inflammation and cancer progression, together with NF- κ B, can transcriptionally upregulate telomerase levels (Ghosh *et al.* 2012).

It was described that telomerase activity was not only related to transcriptional regulation of *TERT* by NF- κ B but also by post-transcriptional regulation by phosphorylation of *TERT* by Akt kinase (Akiyama *et al.* 2002). In multiple myeloma cells, IL6 and insulin-like growth factor 1 (IGF1) would upregulate telomerase activity without alteration of *TERT* protein expression, and this increase was due to the PI3K/Akt/NF- κ B signalling (Akiyama *et al.* 2002).

Another pathway involved in the regulation of *TERT* is the Wnt/B-Catenin. It was described that *TERT* interacts with BRG1, a SWI/SNF-related chromatin remodelling protein, and activates Wnt-dependent reporters in cultured cells and *in vivo*, where *TERT* physically occupies gene promoters of Wnt-dependent genes (Park *et al.* 2009). Transient activation of Wnt/B-Catenin induced *TERT* mRNA expression and elevated telomerase activity in different cell lines. This activation was dependent on TCF4, as *TERT* is a direct target of B-Catenin TCF4-mediated transcription. TCF4-binding site at the *TERT*

promoter is critical for B-Catenin TCF4-dependent expression regulation (Zhang *et al.* 2012). B-Catenin also regulates *TERT* expression through the interaction with Klf4, a core component of the pluripotency transcriptional network in a mouse intestinal tumour model and in human carcinoma cells (Hoffmeyer *et al.* 2012).

Human and mouse HIV-associated nephropathy (HIVAN) show increased levels of *TERT* and activation of Wnt signalling (Shkreli *et al.* 2012). Either silencing *TERT* expression or inhibition of Wnt signalling, through systemic expression of the Wnt inhibitor Dkk1, in *TERT* transgenic mice results in a marked normalisation of podocytes, rapid cell cycle exit, re-expression of differentiation markers and improved filtration barrier function (Shkreli *et al.* 2012).

In contrast to the previously described results, one study failed to find evidence for physical association of *TERT* with BRG1 or B-Catenin in human breast cancer lines and HeLa cells (Listerman *et al.* 2014). Instead, these authors suggest that anti-FLAG antibody cross-reactivity properties may explain the previously reported interaction of *TERT* with B-Catenin (Park *et al.* 2009). Furthermore, altering *TERT* levels in four different breast cancer cell lines caused minimal and discordant effects on Wnt target and Wnt pathway gene expression (Listerman *et al.* 2014).

Another study described that illegitimate activation of B-Catenin signalling enhances the transformation from immortalisation to malignant growth in human foetal hepatocytes (Wege *et al.* 2011). In this model, telomerase inhibition showed that short-term expansion of transformed clones was not telomerase dependent (Wege *et al.* 2011).

In mice *mTERT*^{+/-} and *mTERT*^{-/-} on the CAST/EiJ genetic background, *TERT* overexpression in some settings may activate the Wnt pathways (Strong *et al.* 2011). However, loss of function in a physiological setting has no apparent effects on Wnt signalling (Strong *et al.* 2011). This deficiency leads to telomere shortening, that limits tissue renewal, and these effects are not telomere-independent functions of *TERT* (Strong *et al.* 2011).

Interaction of *TERT* with other signalling pathways and cellular processes

Besides the major *TERT* regulators and targets previously described, several roles for *TERT* have been described in other signalling pathways and cellular processes. Endoplasmic reticulum (ER) stress transiently activates *TERT* expression in human cancer cell lines and murine primary neural cells. Importantly, depletion of *TERT*

sensitises cells to undergo apoptosis under ER stress, whereas increased *TERT* expression reduces ER stress-induced cell death independent of catalytically active enzyme or DNA damage signalling (Zhou *et al.* 2014). *TERT* exerts its anti-apoptotic action at an early stage of cell death process prior to mitochondrial dysfunction and caspase activation (Fu *et al.* 2000). *TERT* may serve as a neuron survival promoter in the developing brain, and downregulation of *TERT* in the adult brain may contribute to increased neuronal vulnerability in various age-related neurodegenerative disorders (Fu *et al.* 2000).

TERT can be targeted to the mitochondria by an N-terminal leader sequence, and mitochondrial extracts from seven different human cell lines show telomerase activity (Santos *et al.* 2004). The cellular effects of mitochondrial telomerase are controversial. Some authors argue that mitochondrial telomerase increases hydrogen peroxide (H₂O₂)-mediated mitochondrial DNA (mtDNA) damage. Ectopic *TERT* expression in human cells correlates with increase in mtDNA damage after H₂O₂ treatment (Santos *et al.* 2004). The same group later demonstrated that this increase in mtDNA damage after H₂O₂ exposure is dependent on the presence of *TERT* itself (Santos *et al.* 2006). Further experiments using a dominant negative *TERT* mutant show that telomerase must be catalytically active to mediate the increase in mtDNA damage. Mutations in the N-terminal mitochondrial leader sequence of *TERT* cause a complete loss of mitochondrial targeting without affecting catalytic activity. Cells carrying this mutated *TERT* not only have significantly reduced levels of mtDNA damage after H₂O₂ treatment, but strikingly also do not show any loss of viability or cell growth. Thus, the authors proposed that localisation of *TERT* to the mitochondria renders cells more susceptible to oxidative stress-induced mtDNA damage and subsequent cell death. Nuclear targeted *TERT*, in the absence of mitochondrial localisation, is associated with diminished mtDNA damage, increased cell survival and protection against cellular senescence (Santos *et al.* 2006).

By contrast, several reports proposed that *TERT* mitochondrial localisation has a protective role against mtDNA damage. Ahmed and coworkers (Ahmed *et al.* 2008) described that in a mild oxidative stress background, telomerase did not prevent telomere shortening under hyperoxia as it translocated gradually from the nucleus to mitochondria. *TERT* mitochondrial localisation reduced mtDNA damage levels under oxidative stress and improved mitochondrial function, with lower levels

of ROS and with enhanced mitochondrial membrane potential (Ahmed *et al.* 2008). Haendeler and coworkers (Haendeler *et al.* 2009) also reported TERT's protective role in mitochondria in an oxidative stress background. TERT binds to *ND1* and *ND2* genes, protecting against mtDNA damage, reducing mitochondrial ROS, and increasing energy metabolism and conferring higher protection from apoptosis (Haendeler *et al.* 2009).

Singhapol and coworkers (Singhapol *et al.* 2013) also described a correlation between TERT retained within the nucleus and high nuclear DNA damage. The group described a heterogeneous stress response in the exclusion of telomerase from the nucleus in cancer cell populations upon stress application. Cells that excluded telomerase quickly from the nucleus accumulated no or very low amounts of DNA damage, suggesting that mitochondrial telomerase prevents nuclear DNA damage as well as the induction of apoptosis after treatment with H_2O_2 and irradiation (Singhapol *et al.* 2013).

Recently, Muzza and coworkers (Muzza *et al.* 2016) described that in thyroid cancer, namely in PTC, there were no differences in mitochondrial oxidative stress between PTC and normal tissues, supporting the postulated role of mitochondrial TERT in the control of local H_2O_2 production. Although TERT nuclear expression did not differ between PTC and normal tissues, the mitochondrial expression of TERT was significantly higher in tumours. The authors suggest that in thyroid cancer, the elevated levels of ROS could be responsible for the shuttling of TERT from the nucleus to the mitochondria (Muzza *et al.* 2016).

A possible explanation for the opposing results concerning the role of mitochondrial telomerase was given by Gordon and Santos (Gordon & Santos 2010) in their 2010 review on the subject. The authors suggest that controversial results may reflect differences in the cellular backgrounds, antioxidant defences and/or in the dose/length of the stress (Gordon & Santos 2010).

Additionally, *TERT* has been described to have influence in several other molecules and pathways, which modify responses to inflammation, cell death, apoptosis and DNA damage responses. For instance, in a comparison between normal and *TERT* immortalised fibroblasts using a cDNA microarray, Lindvall and coworkers (Lindvall *et al.* 2003) verified that *TERT* immortalised cells had 172 differentially expressed genes; one of them is epiregulin, a potent growth factor associated with cancer. The results suggest that both activation of telomerase and subsequent induction of epiregulin are required for a sustained cell proliferation (Lindvall *et al.* 2003).

Young and coworkers suggested two epigenetics mechanisms for the maintenance of a young phenotype in normal human fibroblasts with *TERT* re-expression. The first was the freezing of the epigenomic state of young proliferating cells by the stabilisation of DNA methylation; the second, the maintenance of low levels of the cell cycle inhibitor p21, mediated at least partially by DNMT1's transcriptional repressor activity (Young *et al.* 2003).

In human cervical cancer cell lines, p27/kip1, a tumour suppressor protein, can inhibit *TERT* mRNA expression and telomerase activity through post-transcriptional upregulation by interferon- γ (IFN γ)/IRF1 signalling. This suggests that p27 may have the function of tumour suppressor by inhibiting *TERT* expression (Lee *et al.* 2005). It was also described that *TERT* overexpression upregulated the expression and transcriptional activity of a key cell cycle regulator, cyclin D1, in human prostate epithelial cell lines. This means that *TERT* could have a role in the modulation of cyclin D1 expression (Jagadeesh & Banerjee 2006). *TERT* is capable of activating the transcription of vascular endothelial growth factor (*VEGF*) in WI-38 and HeLa cells, this activation being independent of telomerase activity and telomere maintenance (Zhou *et al.* 2009).

Suppression of *TERT* expression abrogates the cellular response to DNA double-strand breaks. Loss of *TERT* does not alter short-term telomere integrity but instead affects the overall configuration of chromatin. Cells lacking *TERT* exhibit increased radiosensitivity, diminished capacity for DNA repair, fragmented chromosomes, demonstrating that loss of *TERT* impairs the DNA damage response (Masutomi *et al.* 2005).

Role of *TERT* in proliferation, invasion and tumourigenesis

The first evidence that *TERT* by itself was directly involved in tumourigenesis was reported in 2002 (Artandi *et al.* 2002). Transgenic mice expressing *TERT* at high levels had increased telomerase enzymatic activity in several tissues, such as mammary gland, splenocytes and cultured mouse embryonic fibroblasts. This was associated with spontaneous development of mammary intraepithelial neoplasia and invasive mammary carcinomas in a significant proportion of elderly females. This suggested that *TERT* expression could promote the development of spontaneous cancer even in the setting of ample telomere reserve (Artandi *et al.* 2002). In another experiment, using GM847 cell line that presents alternative lengthening of telomeres (ALT) phenotype, the authors showed that expression of oncogenic RAS failed to fully transform the

cells. Nonetheless, with ectopic expression of *TERT*, the cells acquired a tumourigenic phenotype, suggesting that *TERT* had an additional function required for cellular transformation and not depending on its ability to maintain telomeres (Stewart *et al.* 2002). Following this line of reasoning, several studies confirmed the capacity of *TERT* to cooperate with other factors to promote tumourigenesis, for instance, when reduced viability and increased cancer incidence was noted in K5-*TERT* mice with a p53 (+/–) genetic background, indicating that telomerase could cooperate with loss of p53 function in inducing tumourigenesis in ageing organisms (Gonzalez-Suarez *et al.* 2002).

Okamoto and coworkers identified *TERT* as a necessary component to maintain a stem cell state of the tumour-initiating cells (TIC) (Okamoto *et al.* 2011). The formation of a complex *TERT*-GNL3L/NS, which are proposed as markers for TICs in highly aggressive tumours, seems to contribute directly to cancer stem cell formation (Okamoto *et al.* 2011).

With the use of a panel of *TERT* mutants transduced into HMECs, it was shown that *TERT* had at least four biological actions that were genetically separable and functionally independent (Mukherjee *et al.* 2011). *TERT* could enhance independently cell proliferation, telomere elongation, cellular lifespan extension and regulate DNA damage responses (Mukherjee *et al.* 2011).

Using telomerase overexpressing transgenic mice, Gonzalez-Suarez and coworkers showed that the epidermis of the mice was highly responsive to the mitogenic effects of phorbol esters (Gonzalez-Suarez *et al.* 2001). These mice were also more susceptible than the wild-type mice for the development of skin tumours upon chemical carcinogenesis induction (Gonzalez-Suarez *et al.* 2001). Curiously, telomerase transgenic mice showed an increased wound healing rate than wild-type mice (Gonzalez-Suarez *et al.* 2001).

Ectopic expression of telomerase in HMECs leads to an increased ability to proliferate. This expression results in a diminished requirement for exogenous mitogens and correlates with telomerase-dependent induction of genes that promote cell growth. Inhibition of one of these genes, for instance of the epidermal growth factor receptor (*EGFR*), reverses the enhanced proliferation caused by telomerase (Smith *et al.* 2003).

Several other studies implied the role of *TERT* in the enhancement of proliferation, in mouse skin epithelium, specifically in the hair follicle bulge region (Sarin *et al.* 2005), keratinocyte proliferation in skin and activation of hair follicles stem cells (Flores *et al.* 2005).

Interestingly, several splicing variants of *TERT* were described in human and in chicken (Kilian *et al.* 1997, Wick *et al.* 1999, Hisatomi *et al.* 2003, Chang & Delany 2006, Saeboe-Larsen *et al.* 2006, Amor *et al.* 2010). In 2012, another human variant was described, containing an in-frame deletion, which removed exons 4 through 13 ($\Delta 4$ –13), encoding the catalytic domain of telomerase (Hrdlickova *et al.* 2012). This variant was expressed in telomerase-negative normal cells and tissues, as well as in telomerase-positive cell lines (Hrdlickova *et al.* 2012). Its overexpression leads to elevated proliferation rates of several cell types, without affecting telomerase activity, by stimulation of Wnt signalling (Hrdlickova *et al.* 2012). Another study of splice variants described that the variant termed alpha+beta– or beta-deletion is highly expressed in stem and cancer cells (lacking most of the reverse transcriptase domain) and confers a growth advantage to breast cancer cells independent of telomere maintenance, by inhibiting cisplatin-induced apoptosis (Listerman *et al.* 2013).

In a mouse model with constitutive expression of *TERT* targeted to thymocytes and peripheral T cells, *TERT* was associated not only with the development of spontaneous T cell lymphoma but also to its dissemination (Canela *et al.* 2004). The T cell lymphomas in these mice were more disseminated than those in the age-matched wild type, affecting both lymphoid and non-lymphoid tissues, whereas the controls had only the lymphoid tissues affected. These results support the idea that *TERT* has a role in the promotion of tumour progression and dissemination independent of the role in telomere length maintenance (Canela *et al.* 2004).

In gastric cancer, *TERT* was implicated in metastasis in several studies: (i) *TERT*-overexpressing cells had higher levels of Mac2-binding protein (Mac2BP) (Park *et al.* 2007), a tumour antigen implicated in metastasis; (ii) *TERT* binds to c-MYC and recruits the complex to heparanase promoter to upregulate heparanase expression promoting invasion and metastasis of gastric cancer cells; furthermore, *TERT*-activated Wnt/B-Catenin signalling promotes c-MYC expression, which could in turn activate *TERT* transcription and expression in a positive feedback loop (Tang *et al.* 2016); (iii) finally, it has been shown that *TERT* regulation of ITGB1 in the MDM2-FOXO3a-ITGB pathway is able to promote gastric cancer invasion (Hu *et al.* 2015).

In an established *TERT*-overexpressing immortalised cell line (IHOK/*TERT*), *TERT* was responsible for the upregulation of the cysteine protease cathepsin D through the regulation of EGT1 that activates invasiveness and

cancer progression (Park *et al.* 2016). Downregulation of *TERT* during interferon gamma (IFN γ) treatment in human glioblastoma SNB-19 and LN-18 cell lines, effectively inhibited angiogenesis and tumour progression (George *et al.* 2009).

Another study established the relationship between the activation of NF- κ B signalling pathway and upregulation of the metalloproteinases (MMPs) 1, 3, 9 and 10 with enhancement of invasion. Ectopic expression of *TERT* and its catalytic mutant *TERT* K626A induced cancer cell invasion, MMP 9 mRNA expression and promoter activity in an NF- κ B-dependent manner, regulating several target genes of this signalling pathway (Ding *et al.* 2013).

It seems that *TERT* has a role in the epithelial-mesenchymal transition (EMT) process. *TERT* has a pivotal role in invasion and metastasis in stress hormone norepinephrine (NE)-induced ovarian cancer (Choi *et al.* 2015). NE induces *TERT* expression, Slug expression and ovarian cancer cell EMT and invasion (Choi *et al.* 2015). In colorectal cancer cell lines HCT116 and SW480, *TERT* and ZEB1 form a complex, which directly binds to the E-cadherin promoter, inhibiting E-cadherin expression and promoting EMT, thus promoting metastasis (Qin *et al.* 2016). In gastric cancer, *TERT* cooperates with transforming growth factor B1 (TGFB1) and B-Catenin in the promotion of EMT and stemness of gastric cancer cells (Liu *et al.* 2013c).

The main interactions of *TERT* with proliferation, invasion and metastasis are summarised in Table 2.

Functional effects of *TERT* promoter mutations in tumours and cell lines

TERT promoter mutations add a new dimension in the regulation of the *TERT* gene, due to the creation of new consensus binding sites for transcription factors. In the absence of mutations, and as discussed previously, it was shown that *TERT* can affect several pathways and is involved in tumorigenesis, proliferation, invasion and metastasis. The challenge, now, is to clarify how much these mutations can affect *TERT* interaction with other transcription factors and signalling pathways. As result of such, it would be interesting to understand how such mutations contribute through telomerase activity towards an immortalisation role. The main effects of *TERT* promoter mutations are summarised in Table 3.

Using the new tool, CRISPR/Cas9, to study the recurrent mutations in the *TERT* promoter, Xi and coworkers (Xi *et al.* 2015) verified that reverting the -124C>T mutation in a urothelial cancer cell line leads

to decreased telomerase levels, telomere shortening and reduced proliferation rate (Xi *et al.* 2015). These results suggest the causality of the mutation for telomerase activity, telomere length maintenance and cell growth rate (Xi *et al.* 2015).

Another study described similar results showing that *TERT* promoter mutations abrogate *TERT* silencing and impairs telomere shortening (Chiba *et al.* 2015). The authors proposed that cells with the mutant promoter fail to repress transcription of *TERT* as they differentiate, showing high levels of *TERT* expression, telomerase activity and aberrant long telomeres (Chiba *et al.* 2015). This suggests that *TERT* promoter mutations are sufficient, at least in certain conditions, to overcome the proliferative barrier imposed by telomere shortening without additional tumour-selected mutations, meaning that *TERT* promoter mutations can lead to cellular immortalisation and tumorigenesis of incipient cancer cells (Chiba *et al.* 2015).

In a study using 23 urothelial cancer cell lines, Borah and coworkers confirmed that *TERT* promoter mutations correlate with higher levels of *TERT* mRNA, *TERT* protein, telomerase enzymatic activity and telomere length (Borah *et al.* 2015). Furthermore, *TERT* promoter mutations also correlate with worse patient outcome and reduced disease-specific survival in two independent patient cohorts (Borah *et al.* 2015).

In bladder cancer, Li and coworkers (Li *et al.* 2015a) described that the -124C>T mutation frequently occurs in the cancer stem cells, whereas the insertion of this mutation in normal bladder cells leads to *TERT* overexpression and cellular transformation. The reversion of the -124C>T mutation in bladder cancer stem cells can abolish *TERT* expression to basal levels and obliterate tumour formation. On the other hand, the presence of the -124C>T mutation enhances *TERT* expression and telomerase activity, these features being consistent with clinical severity and prognosis of bladder cancer (Li *et al.* 2015a).

The functionality of these mutations is now starting to be unravelled; Bell and coworkers (Bell *et al.* 2015) proposed in a glioblastoma cell line model how these mutations can affect *TERT* reactivation through the binding of ETS transcription factors. They identified multimeric GA-binding protein (GABP) transcription factor as the critical ETS transcription factor activating *TERT* expression in the presence of the -124C>T and -146C>T mutations (Bell *et al.* 2015). Although many ETS transcription factors can bind to similar DNA sequence motifs, GABP seems to be able to bind to neighbouring ETS

Table 2 Summary of interactions of TERT with proliferation, invasion and tumourigenesis.

Cell effects	TERT status	Interaction effects	References
Tumourigenesis and cell proliferation	TERT expression	Cellular transformation Tumourigenic phenotype Cancer stem cell formation Susceptibility to develop cancer	(Artandi <i>et al.</i> 2002) (Stewart <i>et al.</i> 2002) (Liu <i>et al.</i> 2013c) (Okamoto <i>et al.</i> 2011) (Gonzalez-Suarez <i>et al.</i> 2001)
	TERT expression	Increased ability to proliferate	(Flores <i>et al.</i> 2005) (Gonzalez-Suarez <i>et al.</i> 2001) (Lindvall <i>et al.</i> 2003) (Sarin <i>et al.</i> 2005) (Smith <i>et al.</i> 2003)
	TERT expression	Low levels of p21 Freezing of the epigenomic state of young proliferative cells	(Young <i>et al.</i> 2003)
	TERT expression	Upregulation of cyclin D1 Cancer progression	(Jagadeesh and Banerjee 2006)
	TERT expression	P53 ^{+/−} genetic background Increased tumourigenesis in aging organisms	(Gonzalez-Suarez <i>et al.</i> 2002)
Tumourigenesis and cell proliferation	TERT splice variants	Growth advantage Elevated proliferation rates	(Listerman <i>et al.</i> 2013) (Hrdlickova <i>et al.</i> 2012)
Tumourigenesis and cell proliferation	TERT suppression	Elevated p27 levels Inhibition of TERT mRNA expression and telomerase activity	(Lee <i>et al.</i> 2005)
Cell death	TERT expression	Reduction ER-stress induced cell death or DNA damage Anti-apoptotic action at an early stage of cell death Neuron survival promoting function in the developing brain	(Zhou <i>et al.</i> 2014) (Fu <i>et al.</i> 2000)
		Increased susceptibility to mtDNA damage	(Santos <i>et al.</i> 2004) (Santos <i>et al.</i> 2006)
Cell death	Mitochondrial TERT localization	Reduction of mtDNA damage Improvement of mitochondrial function	(Ahmed <i>et al.</i> 2008) (Haendeler <i>et al.</i> 2009) (Singhapol <i>et al.</i> 2013)
	Mitochondrial TERT localization	Higher protection from apoptosis Prevention of nuclear DNA damage	
Cell death	Nuclear TERT localization	Diminished mtDNA damage Increase cell survival Protection against cellular senescence	(Santos <i>et al.</i> 2004) (Santos <i>et al.</i> 2006)
		High DNA damage Promotion of invasion and metastatisation Inhibition of E-cadherin expression Induction of EMT and stemness of cells Spontaneous development and dissemination of T-cell lymphoma	(Singhapol <i>et al.</i> 2013) (Ding <i>et al.</i> 2013) (Hu <i>et al.</i> 2015) (Park <i>et al.</i> 2007) (Park <i>et al.</i> 2016) (Tang <i>et al.</i> 2016) (Qin <i>et al.</i> 2016) (Choi <i>et al.</i> 2015) (Liu <i>et al.</i> 2013c) (Canela <i>et al.</i> 2004) (George <i>et al.</i> 2009)
Invasion and metastatisation	TERT expression		
Invasion and metastatisation	TERT downregulation	Inhibition of angiogenesis and tumour progression	

motifs as a heterotetrameric complex, using the tandem flanking native E26 transforming-specific motifs. Bell and coworkers proposed that *TERT* promoter mutations cooperate with both of these native ETS sites to recruit GABP and reactivate *TERT* transcription (Bell *et al.* 2015).

Another study in glioblastoma, reported that although the −124C>T and −146C>T mutations create similar

binding motif sequences, they are distinct functionally (Li *et al.* 2015b). The −146C>T creates a specific binding site for p52 being driven by non-canonical NF-κB signalling pathway (Li *et al.* 2015b). Li and coworkers also elucidate that the binding of ETS alone is ineffective in the activation of *TERT* transcription in the −146C>T mutation (Li *et al.* 2015b). The stimulation of the non-canonical NF-κB

Table 3 Summary of functional effects of TERT promoter mutations in tumours and cell lines.

Alteration	Cell effect	References
Presence of the mutations	Higher levels of TERT mRNA Increase of telomerase activity Impair of telomere shortening transcription Abolishment of epigenetic silencing: mark of active chromatin Mediation of long-range chromatin interaction Enrichment of active histone marks	(Vinagre <i>et al.</i> 2013) (Borah <i>et al.</i> 2015) (Li <i>et al.</i> 2015a) (Heidenreich <i>et al.</i> 2015) (Stern <i>et al.</i> 2015)
Presence of the mutations	Patients poorer outcome Reduced disease specific survival	(Griewank <i>et al.</i> 2014) (Nagore <i>et al.</i> 2016) (Populo <i>et al.</i> 2014) (Batista <i>et al.</i> 2016) (Mosrati <i>et al.</i> 2015) (Simon <i>et al.</i> 2015) (Yuan <i>et al.</i> 2016) (Melo <i>et al.</i> 2014) (Vinagre <i>et al.</i> 2013) (Bell <i>et al.</i> 2015) (Li <i>et al.</i> 2015b)
–124C>T mutation: Binding of GABPA –146C>T mutation: Binding of NF-κB p52	Reactivation of TERT Interaction with ETS factors Activation of the non-canonical NF-κB pathway Enhanced tumorigenesis Enhanced proliferation Increase of telomerase activity	
Insertion of the mutation	Abrogation of TERT transcription silencing Increase of telomerase activity Impair of telomere shortening Obliterate tumour formation GABPA binding Upregulation of TERT via long-range interactions Acquisition of active histone marks POL2 recruitment	(Chiba <i>et al.</i> 2015) (Li <i>et al.</i> 2015a) (Akincilar <i>et al.</i> 2016)
Reversion of the mutation	Decrease of telomerase activity Telomere shortening Reduced proliferation rate Abrogation of GABPA binding and long-range interaction Depletion of active histone marks Suppression of TERT transcription	(Xi <i>et al.</i> 2015) (Akincilar <i>et al.</i> 2016)

pathway selectively induces *TERT* mRNA expression in –146C>T mutant cells, in which the mutation creates a binding site specific for p52 that interacts with ETS factors ETS1/2 to mediate *TERT* reactivation (Li *et al.* 2015b). This study also reports that there is an enhanced tumorigenic and proliferative effect due to NF-κB activation by the increase of telomerase activity dependent on the existence of –146C>T mutation (Li *et al.* 2015b). The results of this work show for the first time the role of non-canonical NF-κB pathway in tumorigenesis and also elucidate a possible mechanism for *TERT* reactivation in cancers with –146C>T mutation (Li *et al.* 2015b).

Stern and coworkers (Stern *et al.* 2015) described another mechanism by which the presence of *TERT*

promoter mutations can cause an epigenetic switch and monoallelic expression (Stern *et al.* 2015). They reported that RNA polymerase II had a strong preference to occupy the mutant –124C>T *TERT* promoter that exhibited the H3H4me2/3 mark of active chromatin and recruited the GABPA/B1 transcription factor. In contrast, the wild-type allele retained the H3K27me3 mark of epigenetic silencing. Stern and coworkers also (Stern *et al.* 2015) claimed that the *TERT* promoter mutations cause the mutated allele to remain selectively active, whereas the other allele becomes repressed (Stern *et al.* 2015).

Recently, Akincilar and coworkers reported in several cell lines that the mutated *TERT* promoter recruitment of GABPA mediates long-range chromatin interaction

Figure 1

Representation of multiple interactions of TERT with several signalling pathways. TERT interaction with PI3-Akt, β -Catenin, c-MYC and NF- κ B signalling pathways leads to increased proliferation, resistance to cell death, inflammation, invasion and metastasis. Repression (p21, p27 and p53) or overexpression (Cyclin D1) of cell cycle regulators by TERT leads to higher levels of proliferation and cell death resistance, by interfering with DNA repair checkpoints. It remains to be further elucidated if TERT localization in the mitochondria, nucleus or endoplasmic reticulum leads to higher/lower levels of mtDNA and nDNA damage and cell death. TERT inhibits cell adhesion molecules, such as E-Cadherin, which gives cell higher capacities to invade and metastasize.

and enrichment of active histone marks, activating *TERT* transcription (Akincilar *et al.* 2016). Reversal of the *TERT* promoter mutations, or deletion of its long-range interacting chromatin, abrogates GABPA binding and long-range interactions, leading to the depletion of active histone marks, loss of POL2 recruitment and suppression of *TERT* transcription. In contrast, the *de novo* insertion of the mutation in the *TERT* promoter allows GABPA binding and upregulation of *TERT* via long-range interactions, acquisition of active histone marks and subsequent POL2 recruitment (Akincilar *et al.* 2016).

Conclusions

TERT promoter mutations represent a possible mechanism for telomerase reactivation and have been pointed so far

as one of the most common events in several human cancers. Additionally, *TERT* has been described as having a key role in several signalling pathways that are frequently altered in cancer (Fig. 1).

Associations have been made in several cancers between the presence of *TERT* promoter mutations, *TERT* mRNA expression levels and aggressive clinico-pathological features leading to a poorer outcome. Nevertheless, the biological bridge between the presence of *TERT* promoter mutations and the aggressive/invasive features displayed by the tumours remains partially unidentified. It is necessary to further understand the biological role of *TERT* promoter mutations in cancer, beyond the primordial mechanism of immortalisation, the telomere maintenance. One of the first steps is to understand the background of these mutations, i.e., how

these mutations reactivate *TERT* transcription, what are their effects on epigenetic regulation of the promoter, which transcription factors are able to bind to the new consensus site, and which regulators and targets of *TERT* are affected by the occurrence of these mutations. These mutations can also represent a mechanism that can interfere in *TERT* localisation within the cell, which could lead to mtDNA and nDNA damages as well as to metabolic alterations in cancer cells. In common with these scenarios we must have tumour cells with a genetic background prone to the expression of transcription factors for the newly generated binding sites. The presence of the mutations without the adequate repertoire of transcriptions factors would be redundant, and this pre-conditioned background could also be one of the reasons why *TERT* promoter mutations represent a growth advantage for certain cell types, i.e., tissue specificity.

Several questions remain unanswered in this field that has been awaked recently. The understanding of the presence of these mutations in the promoter could be one of the first steps to understand telomerase reactivation beyond its role in cell immortalisation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

All authors were involved in the manuscript preparation.

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