

REVIEW

## Chromatin and epigenetic regulation of the telomerase reverse transcriptase gene

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### ABSTRACT

**Telomerase expression and telomere maintenance are critical for long-term cell proliferation and survival, and they play important roles in development, aging, and cancer. Cumulating evidence has indicated that regulation of the rate-limiting subunit of human telomerase reverse transcriptase gene (*hTERT*) is a complex process in normal cells and many cancer cells. In addition to a number of transcriptional activators and repressors, the chromatin environment and epigenetic status of the endogenous *hTERT* locus are also pivotal for its regulation in normal human somatic cells and in tumorigenesis.**

**KEYWORDS** telomerase, transcriptional regulation, chromatin, epigenetic regulation, cancer

### INTRODUCTION

Telomeres serve as essential protective caps of linear chromosomal ends in all eukaryotic cells and their maintenance is indispensable for indefinite proliferative potential. The telomeric TTAGGG repeats are replenished by telomerase (Morin, 1989), a ribonucleoprotein complex that consists of a catalytic reverse transcriptase protein subunit (TERT) (Harrington et al., 1997; Lingner et al., 1997; Meyerson et al., 1997), an RNA template (TERC) (Shippen-Lentz and Blackburn, 1990; Blasco et al., 1995; Collins, 2000), dyskerin (Cohen et al., 2007), and other accessory proteins (Venteicher et al., 2009). In most human tissues, telomerase is either absent or expressed at extremely low levels (Wright et al.,

1996; Masutomi et al., 2003). Consequently, somatic cells suffer telomere attrition upon successive divisions and undergo a crisis (defined as massive cell death) or a permanent cell cycle arrest (replicative senescence) at the end of their lifespan, namely the Hayflick limit.

Telomere maintenance by telomerase plays an important role in human aging and tumorigenesis. Although telomerase is barely detectable in most adult human cells, it is expressed in over 90% of immortal cell lines and cancers (Kim et al., 1994; Shay and Bacchetti, 1997; Hahn et al., 1999b). Telomerase knockout mice (*mTERC<sup>-/-</sup>*) display impairment in organs consisting of highly proliferative tissues and exhibit premature aging (Blasco et al., 1997; Rudolph et al., 1999). Whereas the RNA subunit, TERC, is abundant in most tissues (Blasco et al., 1996), TERT expression is the rate-limiting step in telomerase expression. The level of *hTERT* mRNA is tightly regulated and correlated with telomerase activity (Meyerson et al., 1997; Nakamura et al., 1997). It has been demonstrated that ectopic *hTERT* expression in many cell types results in telomere stabilization, immortalization, and tumorigenic conversion of pre-neoplastic human cells (Bodnar et al., 1998; Counter et al., 1998; Hahn et al., 1999a; Zhu et al., 1999). In the past decade, regulation of the *hTERT* gene has been a focus of many studies. It has been revealed that the *hTERT* promoter is regulated by many transcription factors and signaling pathways. Since these research progresses have been discussed in a number of recent review articles (Ducrest et al., 2002; Janknecht, 2004; Liu et al., 2004; Dwyer et al., 2007; Kyo et al., 2008), we will emphasize on the epigenetic and chromatin mechanisms of *hTERT* regulation in this review.

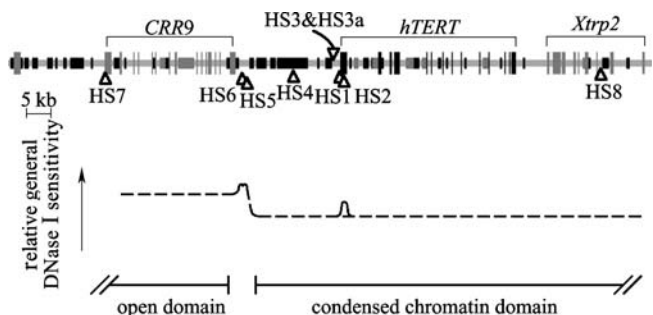
**EXPRESSION OF THE ENDOGENOUS *hTERT* GENE AND ITS TRANSGENIC MODELS**

The *hTERT* gene undergoes repression as embryonic cells differentiate into adult somatic cells. At early embryonic stages, telomerase activity is present at a high level in many tissues (Wright et al., 1996). From the neonatal period onward, this activity can no longer be detected (Kim et al., 1994; Wright et al., 1996), except in some highly proliferative tissues, such as hematopoietic cells and cells of the basal layer of epidermis (Weng et al., 1996; Yasumoto et al., 1996). In addition, the *hTERT* gene is also expressed in stem cells of different types (Chiu et al., 1996).

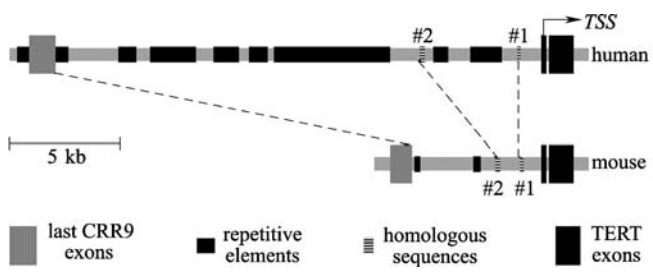
Significant differences exist in telomerase regulation and telomere length control among different species. Although telomere shortening functions as a mitotic clock for cellular senescence in human cells, telomere-mediated replicative aging does not appear to be a critical controlling mechanism for the proliferation of mouse cells (Forsyth et al., 2005). Whereas the *hTERT* gene is tightly repressed in most human somatic tissues (Kim et al., 1994; Shay and Bacchetti, 1997), telomerase expression in mice is less restricted, with many tissues expressing significant levels of mTERT mRNA

(Prowse and Greider, 1995; Greenberg et al., 1998; Martin-Rivera et al., 1998; Horikawa et al., 2005). This difference likely contributes to the much longer telomeres in mouse cells and to some of the distinct characteristics of tumor development in mice than in humans.

The *TERT* locus and its neighboring loci are arranged in the same order in humans and mice (Fig. 1). The upstream *CRR9* gene is ubiquitously expressed in all cell lines and tissues that have been examined (Wang et al., 2004), whereas the downstream *Xtrp2* gene is expressed exclusively in kidney (Nash et al., 1998). Though exon sequences of the *TERT* genes are conserved between the two species, flanking sequences are highly diverged (Greenberg et al., 1999). In particular, the intergenic regions between the *CRR9* and *TERT* loci are 23 kb and 6 kb in human and mouse genomic sequences, respectively (Fig. 2). This size difference is largely attributable to the presence of abundant Alu-elements and other human-specific repetitive sequences, which accounted for about half of the 23-kb sequence in intergenic region. It would be reasonable to speculate that such human-specific repetitive elements are more prone to form repressive chromatin structure in human cells than in mouse cells. Indeed, the intergenic region is highly resistant



**Figure 1.** A schematic illustration of the genomic structures and chromatin conformations around the *hTERT* locus. The top part is a display of the genomic region containing the *hTERT*, *CRR9*, and *Xtrp2* loci. Exons are designated as tall vertical rectangles and lines. Short black rectangles represent repetitive elements and short gray rectangles are mini-satellite sequences. The positions of DHSs are indicated by triangles. The lower part summarizes the general DNase I sensitivity of the genomic regions around the *hTERT* locus in human somatic cells and cell lines that have been examined (Wang and Zhu, 2004; Wang et al., 2009).



**Figure 2.** The comparison of 5' intergenic regions of the *hTERT* and *mTERT* loci. #1 and #2 are human and mouse homology regions. TSS, transcription start site.

to nuclease digestion in both telomerase-positive and negative human cells (Wang and Zhu, 2004; Wang et al., 2009) (Fig. 1). Therefore, it is likely that the intergenic regions upstream of the TERT promoters are important for the differential regulation of *TERT* genes.

Studies on transgenic mouse lines containing human genomic sequences indicated that *cis* elements were important for species-specific regulation of the hTERT promoter. The expression profile of a LacZ reporter controlled by an 8-kb hTERT promoter fragment in transgenic mice was overall similar to that of the *hTERT* gene, albeit with variations among transgenic lines (Ritz et al., 2005). Horikawa et al. also reported a transgenic mouse strain with a BAC containing the *hTERT* gene, an 11-kb upstream region, and a 1.2-kb downstream sequence. The hTERT expression profile in this transgenic mouse line was very similar to the endogenous *hTERT* gene, with little expression in most adult somatic tissues, except testis, ovary, and thymus (Horikawa et al., 2005). Recent data from our transgenic mouse model indicated that an hTERT reporter from an integrated BAC construct, including the entire *hTERT* locus and its upstream and downstream neighbors, *CRR9* and *Xtrp2* loci, recapitulated the regulation of the endogenous *hTERT* gene in human tissues (unpublished results). Taken together, these results demonstrated that (1) the BAC constructs contained sufficient *cis* elements for the human-specific developmental programming of the *hTERT* gene; and (2) mouse cells contained all the *trans*-acting factors involved in the developmental regulation of the *hTERT* gene.

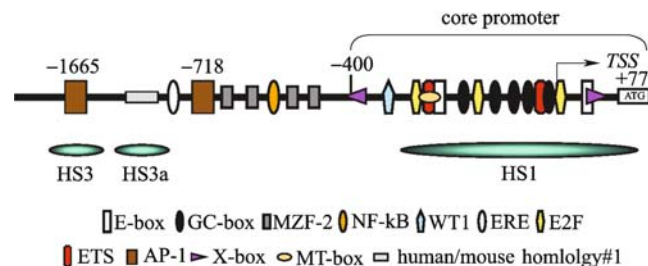
**REGULATION OF THE hTERT PROMOTER FUNCTION**

Studies have indicated that regulation of hTERT transcription is the primary control mechanism for regulating telomerase activity during development and cellular immortalization, although post-transcriptional events, such as alternatively spliced hTERT messages and hTERT protein phosphorylation, have also been reported (Kang et al., 1999; Aisner et al., 2002). The core region of the hTERT promoter contains a number of consensus sequences for known transcription

factors that are likely involved in its regulation, including E-boxes, GC-boxes, Ets and E2F consensus sites (Fig. 3) (Horikawa et al., 1999; Wick et al., 1999; Wu et al., 1999).

It was reported that overexpression of the proto-oncogene c-Myc, which bound to the E-boxes, activated hTERT expression in normal cells (Horikawa et al., 1999; Takakura et al., 1999; Wu et al., 1999). Several other transcription factors, including Sp1, ER, Ets, AP1, E2Fs, and NF-κB, have also been implicated in hTERT regulation via their consensus binding sites within or near the core promoter (Fig. 3) (Kyo et al., 1999; Kyo et al., 2000; Crowe et al., 2001; Akiyama et al., 2003; Goueli and Janknecht, 2004; Takakura et al., 2005; Xu et al., 2008a). Although these regulatory mechanisms of hTERT expression may be important under specific physiological conditions, none of these factors has been associated with immortalization of somatic cells. Several years ago, Hahn and colleagues reported a low-level hTERT expression in normal human fibroblasts (Masutomi et al., 2003). Although this low level of telomerase was insufficient to prevent telomere shortening, it was important for the structural integrity of telomeres in these fibroblasts. Thus, some of the aforementioned factors may be important to maintain the low-level hTERT transcription and telomere integrity in normal somatic cells.

Several negative regulatory factors have been reported to bind to the hTERT promoter and mediate its repression. The downstream E-box (+ 44 nt, relative to the transcription start site, or TSS, Fig. 3) was proposed to play an important role in both positive and negative regulation of hTERT transcription. This E-box was found to mediate the repression of hTERT promoter by Mad1 and USF1 in several cell types (Günes et al., 2000; Horikawa et al., 2002; Lin and Elledge, 2003). The GC-boxes, binding sites for Sp1/Sp3 transcription factors, were essential for hTERT promoter activity and might also be involved in repression by recruiting HDACs (Wu et al., 1999; Kyo et al., 2000; Won et al., 2002). In addition, other sites, including X-box, E2F, MT-box, WT-1 (Wilm’s tumor 1), and MZF-2 sites, might also mediate the repression of hTERT transcription by different transcriptional repressors (Oh et al., 1999; Fujimoto et al., 2000; Crowe et al., 2001; Xu et al., 2001; Horikawa et al., 2002; Xu et al., 2008b).



**Figure 3. Summary of *cis* elements at the proximal region of the hTERT promoter.** Blue ovals indicate the positions of DHSs in HL60 cells (Wang et al., 2009). The horizontal arrow designates the transcription start site (TSS) and the direction of transcription. Numbers are nucleotide positions relative to the TSS. ATG, hTERT translational initiation codon.

Mechanisms of transcriptional activation and repression may coordinate the regulation of the endogenous hTERT promoter. Since c-Myc is up-regulated in many cancer cells, it is thus likely that c-Myc contributes to telomerase activation during tumorigenesis. However, c-Myc is also expressed in some proliferating normal cells in which telomerase activity is undetectable. As in cancer cells, telomerase and the *hTERT* gene are activated upon the formation of induced pluripotent stem cells (iPS cells) (Takahashi et al., 2007; Yu et al., 2007). Although c-Myc was reported to be one of the four reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) (Takahashi and Yamanaka, 2006), it is dispensable for iPS cell induction (Wernig et al., 2008). In addition, hTERT transcription remained silenced in a subset of partially reprogrammed cells containing the highly expressed c-Myc transgene (Mathew et al., unpublished results). These results indicated that c-Myc expression was neither sufficient nor required for the activation of *hTERT* gene during cellular reprogramming. Therefore, mechanisms of transcriptional repression must be present in normal somatic cells to prevent the *hTERT* gene from being activated (Horikawa et al., 1998; Ducrest et al., 2002; Wang and Zhu, 2003).

### CHROMATIN AND EPIGENETIC REGULATION OF THE *hTERT* GENE

Regulation of gene expression appears to be a complex process, often occurring at multiple levels. On the one hand, transcriptional activation of a promoter involves temporal actions of transcription factors, covalent modifications of core histones by chromatin modifying complexes, and ATP-dependent nucleosomal remodeling at the promoter (de la Serna et al., 2006; Kaeser and Emerson, 2006). On the other hand, distinct and redundant chromatin modifications such as histone deacetylation and nucleosomal remodeling have been implicated in the transcriptional repression of several yeast genes (Zhang and Reese, 2004).

Covalent modifications of core histones, including histone acetylation, methylation, phosphorylation, and ubiquitination, have emerged as major mechanisms for gene regulation (Jenuwein and Allis, 2001). It has been shown that, trichostatin A, a potent inhibitor of classes I and II histone deacetylases (HDACs), induced hTERT transcription in normal cells and telomerase-negative immortal cell lines (Cong and Bacchetti, 2000; Takakura et al., 2001; Hou et al., 2002; Wang and Zhu, 2003; Liu et al., 2004). In addition, hTERT transcription was also associated with hyperacetylation of core histones at the hTERT promoter in several types of cells, as revealed by chromatin immunoprecipitation (ChIP) (Xu et al., 2001; Wang et al., 2007). These results indicated that the hTERT promoter was normally repressed as a result of hypoacetylation of core histones in many human somatic cells.

In addition to histone deacetylation, histone methylation

was also reported to be involved in hTERT regulation. Atkinson et al. showed that the lack of hTERT transcription in telomerase-negative immortal cells was associated with methylation at lysine 9 of histone H3 (H3K9) and lysine 20 of H4 (H4K20), whereas hTERT expression in telomerase-positive cells was accompanied by methylation at lysine 4 of histone H3 (H3K4) (Atkinson et al., 2005). Interestingly, depletion of the histone methyltransferase SMYD3 in tumor cells resulted in a reduction of histone H3K4 methylation at the hTERT promoter, accompanied by down-regulation of hTERT mRNA and telomerase activity, indicating that SMYD3 activated the hTERT promoter by methylating the H3K4 residue (Liu et al., 2007). Furthermore, inhibition of lysine-specific demethylase 1 (LSD1) by either siRNAs or a chemical inhibitor tranylcypromine led to an increase of methylation at H3K4 and an activated hTERT transcription human fibroblasts (Zhu et al., 2008). Most recently, the same group also reported that recruitment of histone demethylase RBP2 by Mad1 was involved in the transcriptional repression of the *hTERT* gene in leukemic HL60 cells (Ge et al., 2009). Finally, Xu and colleagues showed that the mitogen-activated kinase (MAPK) signaling pathway mediated histone H3 phosphorylation is required for the activation of hTERT transcription during cell proliferation in fibroblasts and T lymphocytes (Ge et al., 2006).

In addition to histone modifications, CpG methylation is another important epigenetic mechanism of transcriptional silencing. A CpG island is present at the 5' end of the *hTERT* gene, overlapping with the hTERT promoter (Wick et al., 1999). Although it has been reported that the hTERT promoter was often methylated in human cell lines and cancer cells (Devereux et al., 1999; Dessain, 2000 #357), a clear correlation between its transcriptional activity and methylation profile has not been established. On the one hand, promoter methylation was associated with transcriptional silencing of the *hTERT* gene, as treatment of cells with demethylating agent 5-aza-2'-deoxycytidine resulted in an increase in hTERT transcription in an immortal fibroblast line SUSM-1 (Devereux et al., 1999). In addition, the binding of methyl-CpG-binding domain protein 2 (MBD2) to the hypermethylated hTERT promoter led to hTERT repression in HeLa cells (Chatagnon et al., 2009). On the other hand, DNA hypermethylation was also implicated in the positive regulation of the hTERT promoter (Guilleret et al., 2002) because demethylation in several telomerase-positive tumor cell lines caused reduced hTERT expression and telomerase activity, which were accompanied by telomere shortening (Guilleret and Benhattar, 2003). In this case, CpG methylation likely interfered with the binding of transcriptional repressors and thereby positively regulated the hTERT promoter (Renaud et al., 2005).

The paradox for the conflicting data regarding the role of promoter methylation in hTERT regulation in cancer cells remains to be resolved. The apparent discrepancy might in

part result from the different methods used in various studies. Using methylation-specific PCR and bisulfite sequencing methods, Zinn et al. analyzed the hTERT promoter in breast, lung, and colon cancer cells, and found that most cancer cell lines retained at least one allele with little or no methylation in a small region near TSS, in spite of dense methylation in a region 600 bp upstream of the TSS (Zinn et al., 2007). This study suggested that demethylation at the core promoter region was required for hTERT transcription in tumor cells. Conversely, the hTERT promoter in many normal cells and tissues, in which the hTERT gene was transcriptionally silenced, was either unmethylated or hypomethylated, indicating that developmental hTERT silencing did not require extensive CpG methylation at its promoter (Devereux et al., 1999; Dessain et al., 2000; Guilleret et al., 2002; Wang et al., 2007). Although the role of CpG methylation in hTERT regulation may vary among different cell types, it is possible that most *de novo* CpG methylation at the hTERT promoter was acquired during tumor growth and/or *in vitro* passaging of oncogene-transformed cell lines (Dessain et al., 2000). Consistent with this notion, the CpG island at the hTERT promoter was more extensively methylated in SV40-transformed human fibroblasts than in their parental normal human fibroblasts, although the endogenous hTERT mRNA was detected in neither cell types (unpublished results).

In the past decade, much attention has been focused on the regulation of hTERT promoter. To this date, all *cis* regulatory elements that have been reported to be responsible for hTERT regulation reside in the proximal region of the hTERT promoter. In fact, the hTERT promoter in an episomal state in transient transfection experiments was highly active in both telomerase-positive and -negative cells, comparable to those of SV40 early promoter and CMV promoter (Cong et al., 1999; Ducrest et al., 2001; Wang and Zhu, 2003). In contrast, the expression of endogenous hTERT gene was tightly regulated in these cells. Even in many telomerase-positive cell lines, the endogenous hTERT gene was expressed at relatively low levels, with only a few copies of hTERT mRNA per cell (Ducrest et al., 2001; Yi et al., 2001; Renaud et al., 2003). Although posttranscriptional events, such as alternative RNA splicing events, might contribute to this drastic difference between the activity of an episomal hTERT promoter and its endogenous expression (Aisner et al., 2002), available evidences suggested that distal regulatory elements, in addition to the *cis* elements resided in the hTERT proximal promoter region, also played important roles in regulating the endogenous hTERT gene. Indeed, sequences within the first and second exons of the hTERT gene were found to contain binding sites for CTCF, an abundant and highly conserved nuclear factor that was primarily involved in the global organization of chromatin architecture, suggesting that chromatin environment and epigenetic mechanisms contributed to the regulation of endogenous hTERT promoter in both normal and cancer cells (Renaud et al., 2005).

Consistent with the notion that the chromatin environment is critical for the tight regulation of the hTERT gene in somatic cells, studies from our laboratory revealed that the endogenous hTERT locus was embedded in a condensed chromatin domain that was as resistant to DNase I digestion as the well-studied  $\beta$ -globin loci in normal human fibroblasts and immortal cell lines, including both telomerase-positive and -negative cell lines (Wang and Zhu, 2004). This chromatin domain was at least 100 kb in size, extending from the 5' intergenic region of the hTERT gene to the downstream *Xtrp2* locus (Fig. 1). In telomerase-positive cells, the hTERT transcription was accompanied by the appearance of a major DNase I hypersensitive site (DHS) at the core promoter (Wang and Zhu, 2003, 2004) and several other DHSs downstream and upstream of the core promoter (Wang et al., 2009), suggesting the potential positions for regulatory elements. In telomerase-negative cells, HDAC inhibition by trichostatin A resulted in an opening of this condensed chromatin domain, leading to an activated transcription from the hTERT gene (Wang and Zhu, 2004). These results indicated that the native chromatin environment was critical for the tight regulation of the hTERT gene.

The chromatin environments of the native loci may also contribute to the differential regulation of hTERT and mTERT genes. Both genes were down regulated during cellular differentiation (Wang et al., 2009), yet repression of the hTERT gene was much stronger than that of the mTERT gene. Since transiently transfected hTERT promoters were not repressed in differentiated cells, the stringent hTERT regulation in somatic cells likely depended on the native chromatin environment (Wang and Zhu, 2003; Wang et al., 2009). Interestingly, while the hTERT locus was located within a nuclease-resistant condensed chromatin domain in human cells, a corresponding chromatin domain was not detected for the mTERT locus (Wang et al., 2009). Taken together, these studies indicated that, unlike repression of the mTERT gene, the condensed native chromatin environment of the hTERT locus was central to its silencing during cell differentiation.

## REGULATION OF THE hTERT GENE IN TUMORIGENESIS

Whereas the hTERT gene is repressed in most somatic cells, telomerase activity is detected in the majority of cancer cells (Kim et al., 1994). Several mechanisms may contribute to the hTERT activation in cancer cells. First, transcriptional activators that are expressed in cancer cells may lead to activated transcription from the hTERT gene. For example, it was reported that the hTERT gene was activated by oncoprotein c-Myc, through binding to the E-boxes at the core promoter in normal and cancer cells (Wang et al., 1998; Greenberg et al., 1999). In addition, it was also shown that the hTERT promoter was activated by the viral oncoprotein E6

from a subset of human papillomaviruses (HPVs), by binding to the E-boxes in epithelial cells (Klingelutz et al., 1996; Veldman et al., 2003; Xu et al., 2008b). Second, activation of the *hTERT* gene may result from the loss or inactivation of transcriptional repressors in cancer cells. Transforming growth factor  $\beta$  (TGF- $\beta$ ) family proteins, a group of cytokines that inhibit cell proliferation and promote differentiation in many cell types, have been proposed to initiate a tumor suppressor pathway. An important mechanism of the tumor suppression by TGF- $\beta$  may be through inhibition of the *hTERT* gene transcription (Li and Liu, 2007). It was recently reported that TGF- $\beta$  and related protein BMP7 inhibited hTERT transcription in several cancer cell lines (Li et al., 2006; Cassar et al., 2008). Repression of the hTERT promoter required the TGF- $\beta$  signaling transducer protein Smad3, which translocated to nuclei upon activation of the pathway and bound to the hTERT promoter either directly or through its interaction with c-Myc (Li et al., 2006). Thus, disruption of the TGF- $\beta$  signaling would lead to an activation of the *hTERT* gene in cancer cells (Yaswen and Stampfer, 2002).

In addition to overexpression of transcriptional activators and loss of repressors, available evidences also indicated that activation of the *hTERT* gene in many cancer cells involved additional genetic and epigenetic mechanisms. For example, Bazarov et al. recently reported that c-Myc overexpression was insufficient to induce a significant increase of hTERT transcription (Bazarov et al., 2009). In c-Myc-immortalized human mammary epithelial cells, telomerase activation was accompanied by the amplification of either the whole or a portion of chromosome 5, where the *hTERT* locus is located. Several other reports have also shown that the *hTERT* gene was amplified in many cancer cell lines and primary tumors, including those of brain, breast, cervix, liver, and lung origins (Zhang et al., 2000; Zhang et al., 2002; Takuma et al., 2004), as well as primary and metastatic melanomas (Pirker et al., 2003). However, in the majority of cases, the amplification ratio, the ratio of *hTERT* gene copy number to the number of chromosome 5p, was less than two (Zhang et al., 2000; Pirker et al., 2003; Cao et al., 2008). Because transcription from the endogenous *hTERT* gene was very low in most human somatic tissues (Wright et al., 1996; Ducrest et al., 2001; Horikawa et al., 2005), the modest amplification could not have accounted for the large increases of hTERT mRNA expression in most cancer cells. Indeed, no statistically significant association was found between the copy number of the *hTERT* gene and its expression level or telomerase activity in the studies of a large set of colorectal carcinomas (Palmqvist et al., 2005), suggesting that amplifications of the *hTERT* locus per se was not a direct cause of hTERT activation and additional genetic or epigenetic events were likely involved.

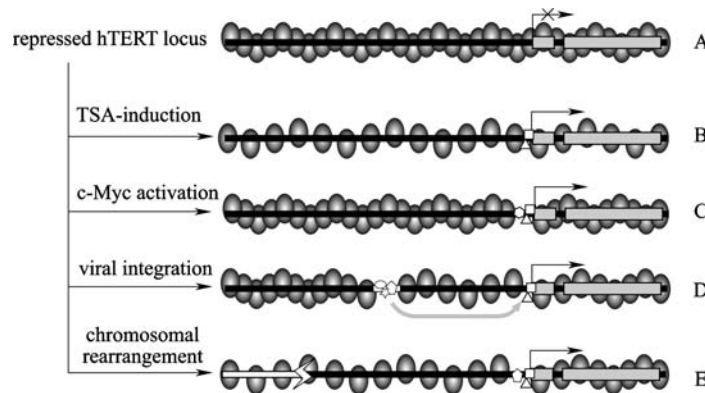
In fact, the hTERT promoter was subjected to genetic alterations in cancer cells. It was shown that viruses

frequently integrated into sequences in the proximity of the hTERT promoter in hepatitis B virus (HBV)-induced hepatocellular carcinoma and HPV-induced cervical cancers (Ferber et al., 2003). Most of these integrations occurred within the hTERT promoter or upstream region and one integration was found in intron 3 without altering the coding sequence, indicating that activation of telomerase by these viral integration promoted tumor progression.

Recently, we also detected that, the *hTERT* gene, located near the telomere of chromosome 5p, was translocated in telomerase-positive cell lines but not in their parental pre-crisis cells and telomerase-negative cell lines (Zhao et al., 2009). Detailed Southern analysis mapped the breakage points to the upstream region of the hTERT promoter, indicating that the *hTERT* gene was the direct target of these chromosomal rearrangements. Moreover, the levels of hTERT mRNA in immortal cell lines correlated inversely with the distance between chromosomal breakage points and the hTERT promoter. Since the *hTERT* locus was embedded in a condensed chromatin domain (Wang and Zhu, 2004), genomic rearrangements would have allowed the hTERT promoter to escape the repressive chromatin environment and thereby activate the telomerase gene, leading to cellular immortalization (Fig. 4).

In the oncogene-induced *in vitro* immortalization models of human fibroblasts, normal fibroblasts were first transformed with the SV40 T antigens, allowing the cells to overcome the M1 senescence and acquire an extended life span (Shay and Wright, 1989; Zhu et al., 1999). These cells continued to proliferate and eventually entered an M2 crisis when their telomeres became critically short and lost the capping function (Blackburn et al., 2000). During the crisis, dysfunctional telomeres formed end-to-end chromosome fusions, initiating breakage-fusion-bridge cycles and consequently a mitotic catastrophe (Counter et al., 1994; Zhu et al., 1999). Only very rarely did cells survive the crisis and became immortal lines (Shay and Wright, 1989; Wang and Zhu, 2003). The chromosomal breakages at the *hTERT* locus and subsequent ligations to heterologous regions by non-homologous end joining (NHEJ) led to nonreciprocal translocations, such as those present in telomerase-positive immortal cells. The very rare cells in which the *hTERT* gene was activated survived this crisis and were ultimately established as immortal cell lines. In fact, the *hTERT* gene was subjected to rearrangements in all telomerase-positive immortal fibroblast lines examined thus far, suggesting that translocation of the *hTERT* gene were caused by telomere-induced crisis (Shay and Wright, 1989; Wang and Zhu, 2003). This might explain the observation that *in vitro* cellular immortalization events are extremely rare and only occur following telomere-induced crisis (Shay and Wright, 1989; Zhu et al., 1999; Maser et al., 2002).

It has been reported that telomere-induced crisis occurred during tumorigenesis and was an important barrier for tumor



**Figure 4. Models of hTERT activation in human cells.** (A) The *hTERT* locus is embedded in a condensed chromatin domain in normal human fibroblasts and pre-crisis cells. As a result, the promoter is inaccessible to transcription activators. (B) Inhibition of HDACs by trichostatin A results in chromatin decondensation and the opening of the hTERT promoter. (C) Overexpression of activators, such as the oncoprotein c-Myc, induces the hTERT transcription. (D) Integration of viral sequences in the proximity of the hTERT promoter leads to transcriptional activation of the *hTERT* gene. (E) Chromosomal translocations allow the hTERT promoter to escape the repressive chromatin environment. Ovals represent nucleosomes. Gray rectangles denote the first and second hTERT exons. Black bars are genomic sequences upstream of the hTERT promoter; white bars are heterologous upstream genomic sequence fused to the translocated *hTERT* allele. Horizontal arrows indicate the TSS and the direction of transcription. White polygons at the hTERT promoter symbolize various transcription activators.

progression (Maser et al., 2002; Greenberg, 2005; Hornsby, 2007). Therefore, activation of the *hTERT* gene by telomere-induced crisis might also take place during tumor development, consistent with the notion that the crisis was a mutational mechanism that generated diverse genetic alterations required for cancer progression (Greenberg, 2005; Hornsby, 2007); one of these alterations could be telomerase activation through rearrangements of the *hTERT* gene.

## CONCLUSIONS

A large body of evidence accumulated from intensive investigations in the last decade has revealed that regulation of the *hTERT* gene is a very complex process. In addition to a number of transcription factors that bind and regulate the hTERT promoter, the repressive chromatin environment and nucleosomal conformation appear to be one of the major mechanisms that tightly suppress the *hTERT* gene in majority of human somatic cells (Fig. 4A). The repressed hTERT promoter can be activated by multiple mechanisms in different cell types. Inhibition of HDACs leads to global histone acetylation and decondensation of the *hTERT* locus (Fig. 4B). Sequence-specific transcription activators, such as c-Myc, may bind to the hTERT promoter and activate its transcription (Fig. 4C), although it was unclear whether c-Myc binding by itself was sufficient to induce hTERT transcription. The hTERT promoter may also be activated by integration of viral sequences at its proximity in some virus-induced cancers (Fig. 4D). In addition, *hTERT* gene amplifications in many cancers has been reported, although multiple copies of

the repressed gene are unlikely to yield sufficient amount of the hTERT transcript. Gene amplifications combined with other genetic or epigenetic mechanisms may lead to a marked activation of hTERT transcription during tumorigenesis. In cancer cells, it may not be the increased copy number of the *hTERT* gene, but the escape of the hTERT promoter from its native repressive chromatin environment, as a result of chromosomal rearrangements, that leads to the significant increases in hTERT transcription (Fig. 4E). Chromosomal translocations of the *hTERT* locus may in fact be an important mechanism of telomerase activation, as it occurs through dysfunctional telomere-induced crisis during the progression of many cancers and telomerase activation is one of the few means that allows cells to escape this crisis.

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## ABBREVIATIONS

ChIP, chromatin immunoprecipitation; DHS, DNase I hypersensitive site; HBV, hepatitis B virus; HDAC, histone deacetylase; HPV, human papillomavirus; iPS cells, induced pluripotent stem cells; LSD1, lysine-specific demethylase 1; MAPK, mitogen-activated kinase; NHEJ, non-homologous end joining; TERT, telomerase reverse transcriptase; TGF- $\beta$ , transforming growth factor  $\beta$ ; TSS, transcription start site; WT-1, Wilm's tumor 1

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