Regulation of hTERT transcription: a target of cellular and viral mechanisms for immortalization and carcinogenesis

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Abstract

A hallmark of human cancer cells is immortal cell growth, which is associated with telomere maintenance by telomerase. The transcriptional regulation of the human telomerase reverse transcriptase (hTERT) gene is a major mechanism that negatively and positively controls telomerase activity in normal and cancer cells, respectively. A growing body of data suggests that various cellular and viral factors and pathways involved in cell senescence, immortalization and carcinogenesis act on the hTERT promoter. The activity of the hTERT promoter is regulated, either directly or through signaling pathways, by oncogene products (e.g., Myc and Ets families) and tumor suppressor proteins (e.g., BRCA1). Endogenous factors involved in the physiological repression of the hTERT gene have also been revealed by chromosome transfer experiments. The integration of viral genomes in the hTERT locus can lead to hTERT activation and telomerase induction. Here, we summarize these findings and pay special attention to recent findings with relevance to the endogenous regulatory mechanisms of hTERT transcription.

Abbreviations: ChIP – chromatin immunoprecipitation; HBV – hepatitis B virus; HIF – hypoxia-inducible factor; HPV – human papillomavirus; hTERT – human telomerase reverse transcriptase; MMCT – microcell-mediated chromosome transfer; RNAi – RNA interference; siRNA – small interfering RNA; TRRAP – transformation-transactivation domain-associated protein.

Introduction

Normal human somatic cells are destined to divide only a limited number of times and eventually cease to divide through a process termed cellular senescence. In contrast, cancer cells often have the potential to divide indefinitely or at least many more times than normal cells (Mathon and Lloyd

2001). This difference in division potential between cancer and normal cells is explained by the presence or absence of a mechanism to maintain telomeres during cell divisions, which usually depends on a telomeric DNA repeat-synthesizing enzyme, telomerase. Thus, telomerase-mediated telomere maintenance is critical for the immortal growth of cancer cells (Bryan and Cech 1999). Conversely,

the tight repression of telomerase in normal cells is responsible for their limited division potential and for cellular senescence, which acts as a tumor suppressive mechanism in humans (Campisi et al. 2001). Human telomerase consists of an RNA component and of multiple protein components. Among these, the expression of the human telomerase reverse transcriptase (hTERT) appears to be a key determinant of the enzymatic activity of human telomerase (Meyerson et al. 1997; Nakamura et al. 1997). While the posttranscriptional and posttranslational modification of hTERT can affect telomerase activity (Aisner et al. 2002), a major contributor to the regulation of telomerase activity is the transcriptional control of the hTERT gene. In many tumors, hTERT expression is specifically localized to the telomerase-positive cancer cells, while it is inactive in most of the surrounding normal cells (Shay et al. 2001). Based on these findings, investigation of activating and repressive mechanisms of hTERT transcription in cancer and normal cells, respectively, has become an area of intense interest in cancer research.

Earlier studies on hTERT transcriptional regulation provided an initial list of candidate hTERT regulators, many of which investigated under experimental settings leading to their overexpression or ectopic expression (summarized in Horikawa and Barrett 2003 and Poole et al. 2001). Candidate activators included ubiquitous transcription factors (e.g., Sp1 and USF), proto-oncogene products (e.g., c-Myc and Ets family), hormone receptors (e.g., estrogen receptor α) and viral oncoproteins (e.g., human papillomavirus E6 protein). Some tumor suppressor proteins or pathways were suggested to repress hTERT transcription (e.g., WT1, p53, Mad1, BRCA1 and TGF- β signaling). However, it is not yet known whether these candidate factors participate in endogenous mechanisms involved in controlling hTERT transcription under physiological conditions. This review essentially summarizes recent studies that have implications in answering this outstanding question. A chromosome transfer-based approach for identifying endogenous hTERT repressors in a physiological setting (vs. overexpression or ectopic expression) is described. In addition to its regulation by trans-acting transcription factors and pathways, the hTERT gene itself can be subjected to genomic rearrangements

resulting in its transcriptional activation in cis. We also discuss the role of tumor microenvironment, such as hypoxia, in the regulation of hTERT expression.

c-Myc-mediated activation of hTERT transcription

When the promoter region of the hTERT gene was cloned by several groups in 1999 (Greenberg et al. 1999; Horikawa et al. 1999; Takakura et al. 1999; Wu et al. 1999), its most interesting feature was the presence of two canonical E-box sequences (CACGTG), which are known to act as potential binding sites for the Myc family of proto-oncogene products. This prompted researchers to examine the role of the Myc family (in particular, the c-Myc protein) in the transcriptional regulation of the hTERT gene. A recombinant c-Myc protein was shown to bind the E-box sequences of the hTERT promoter, and overexpression of the c-Myc protein resulted in the activation of hTERT promoter activity, as measured by a reporter gene assay and/or the induction of the endogenous hTERT mRNA (Greenberg et al. 1999; Horikawa et al. 1999; Takakura et al. 1999; Wu et al. 1999). Taken together with the increased expression of c-Myc in malignancies, these initial findings suggested a role for the c-Myc protein and its binding sites (E-box) in the activation of hTERT and telomerase activity in human cancers (Figure 1). However, because of the use of supraphysiological levels of exogenous c-Myc protein in functional assays and because of the technical difficulties in detecting the DNA-binding activity of the endogenous c-Myc protein and its association with the endogenous hTERT promoter, it was still unclear whether the endogenous c-Myc protein played a physiological role in the activation of the hTERT gene during carcinogenesis. Two experimental methods, which have recently become widely used, helped define the role of the endogenous c-Myc protein in hTERT transcription: chromatin immunoprecipitation (ChIP) assay for examination of protein–DNA interaction *in vivo* (Johnson and Bresnick 2002); and RNA interference (RNAi) for functional knockdown of endogenously expressed proteins (Caplen and Mousses 2003). Xu et al. (2001) showed by ChIP that the *in vivo* binding of c-Myc to the hTERT promoter correlates with the induction of hTERT expression in proliferating

Figure 1. Transcriptional regulation of the hTERT gene. Activating (white background) and repressive (gray background) mechanisms are schematically shown. c-Myc forms a complex with Max and binds to the E-box within the hTERT promoter. This complex is a target of various positive and negative regulatory mechanisms of hTERT transcription. Another oncogene product activating the hTERT transcription is ER81, a member of the Ets family of transcription factor, which mediates the action of oncogenic signaling pathways. Cis-activation by integrated viral genome is also shown. See text for additional details.

HL60 human leukemic cells. A large-scale screen for c-Myc binding sites by means of quantitative ChIP identified a number of *in vivo* c-Myc target genes, including the hTERT gene (Fernandez et al. 2003). In this study, the *in vivo* binding of c-Myc to the endogenous hTERT gene promoter was detected in both leukemic cells and glioblastoma cells expressing either high- or low-levels of c-Myc protein. These findings have confirmed that the endogenous c-Myc protein is bound to the endogenous hTERT promoter in telomerase-expressing human malignant cells. The functional knockdown of c-Myc by RNAi provided evidence that the hTERT promoter-bound c-Myc is required for hTERT transcription. In human cancer cells, down-regulation of the endogenous c-Myc protein using small interfering RNA (siRNA) resulted in a marked decrease in both hTERT mRNA expression and hTERT promoter activity (Koshiji et al. 2004; Yang et al. 2004).

Although the above-mentioned progress shows the importance of c-Myc in maintaining hTERT expression in human cancers, it still remains to be clarified whether a change in the level or transcriptional activity of the c-Myc protein during carcinogenesis is responsible for the transition from hTERT-negative normal cells to hTERT-expressing cancer cells. The *in vivo* binding of c-Myc to the hTERT promoter remains to be compared in normal vs. cancer cells by the ChIP assay. The activation of hTERT transcription by some oncogenic factors (e.g., Aurora-A kinase; Yang et al. 2004) and its repression by TGF- β

signaling (Cerezo et al. 2002) are associated with changes in c-Myc protein levels. Therefore, the total amount of c-Myc protein may be correlative with the binding of c-Myc to the hTERT promoter, and possibly with the activation of hTERT transcription (Figure 1). However, c-Myc level itself does not always correlate with the transcriptional activity of the hTERT promoter: for example, the activation of the hTERT promoter by the human papillomavirus (HPV) E6 oncoprotein is dependent on the E-box element without being associated with an increase in c-Myc protein levels (Gewin and Galloway 2001; Veldman et al. 2001); and the repression of the hTERT promoter by transfer of normal human chromosome 3 in renal cell carcinoma cells (see below for details) similarly required the E-box elements but without a decrease in c-Myc protein levels (Horikawa et al. 2002). It is likely that, in addition to its regulation by the level of total c-Myc protein, modification of c-Myc transactivation function through protein-protein interaction is important for hTERT activation. In case of hTERT activation in normal human keratinocytes by HPV E6 (Veldman et al. 2003), E6 did not alter c-Myc protein levels and similar amounts of c-Myc protein were bound *in vivo* to the hTERT promoter in normal and E6-transduced keratinocytes, suggesting that c-Myc binding is, by itself, insufficient for hTERT activation in this experimental system. Importantly, in the E6-transduced cells expressing hTERT, the E6 protein interacted with the c-Myc protein and was a component of the c-Myc-containing complex

associated with the endogenous hTERT promoter, suggesting a role for E6 in enhancing the trans-activation function of c-Myc (Figure 1).

Activation of hTERT transcription by other oncogene products

c-Myc-independent mechanisms of hTERT transcriptional regulation exist and other oncogene products may also positively regulate hTERT transcription. Recent data suggest that the Ets family of transcription factors functions as an hTERT transcriptional activator (Maida et al. 2002; Takahashi et al. 2003; Xiao et al. 2003; Goueli and Janknecht 2004). The upregulation of Ets transcription factors is observed in breast and other human cancers. Cellular signaling pathways, such as the mitogen-activated protein (MAP) kinase and the PI-3 kinase signaling pathways, converge on the Ets family of factors (Yordy and Muise-Helmericks 2000), suggesting that the many growth factors and cellular stresses acting upon these signaling pathways might also regulate hTERT transcription (for example, the epidermal growth factor in Maida et al. 2002). Goueli and Janknecht (2004) showed that three oncoproteins frequently associated with human cancers (i.e., HER2/Neu, Ras and Raf) can activate hTERT transcription through the MAP kinase pathway and a member of the Ets family of transcription factors, ER81 (Figure 1). In HER2/Neu-overexpressing breast cancer cells, inhibition of HER2/Neu or ER81 protein function suppressed hTERT promoter activity and telomerase activity. Interestingly, overexpression of HER2/Neu, Ras or Raf in combination with ER81 induced endogenous hTERT transcription and telomerase activity in otherwise hTERT/telomerase-negative human fibroblasts. These findings support the notion that ER81 is a transcriptional activator of the hTERT gene that links oncogenic signals to telomerase activation and cell immortalization during the transition from normal cells to cancer cells. Although the binding of ER81 to the Ets consensus sequences within exon 1 and intron 1 of the hTERT gene was shown by electrophoretic mobility shift assay, the *in vivo* binding of endogenous ER81 to the endogenous hTERT gene remains to be compared in normal vs. cancer cells. It should be noted that the ER81 gene itself can

undergo a malignancy-associated genomic rearrangement (chromosome translocation) resulting in the EWS-ER81 fusion protein, as detected in Ewing's sarcomas (Jeon et al. 1995), which may constitutively activate hTERT transcription. Indeed, Takahashi et al. (2003) reported that the fusion proteins formed by the translocation of the EWS gene with the other Ets family members E1AF and FLI1 (EWS-E1AF and EWS-FLI1) can activate hTERT expression and telomerase activity. Another oncogene product that can regulate hTERT expression independently of c-Myc is Bmi-1, a member of the Polycomb family. Dimri et al. (2002) showed that the overexpression of Bmi-1 led to activation of hTERT transcription and induction of telomerase activity in mammary epithelial cells. In addition to its ability to inhibit the p16/Rb and ARF/p53 pathways (Jacobs et al. 1999), the activation of telomerase is likely to represent an additional mechanism by which Bmi-1 contributes to escape from cellular senescence and the immortalization of mammary epithelial cells. Further experiments (e.g., RNAi inhibition of endogenous Bmi-1 in breast cancer cells, and the elucidation of the mechanisms of transcriptional activation) are needed to establish Bmi-1 as an endogenous hTERT activator of physiological importance. The Bmi-1 protein, which is essential for the proliferative capacity of hematopoietic stem cells (Lessard and Sauvageau 2003; Park et al. 2003), may therefore play an important role in regulating hTERT and telomerase expression in stem cells, as well as in cancer cells.

Tumor suppressors and hTERT transcriptional repression

Some tumor suppressor proteins or pathways may have the ability to repress hTERT transcription by affecting the expression or function of the c-Myc protein. The TGF- β /Smad signaling pathway is known to down-regulate c-Myc expression (Chen et al. 2001; Yagi et al. 2002). The activation or suppression of this pathway in human cancer cells results in significant reductions or increases of hTERT expression and telomerase activity, respectively (Yang et al. 2001). Although it remains to be determined whether the inactivation of this pathway (e.g., through mutations in genes encoding TGF- β receptors or Smad proteins) is directly

involved in the activation of hTERT during malignant transformation, the TGF- β /Smad signaling pathway is likely to affect hTERT expression through its ability to downregulate c-Myc protein levels (Figure 1). BRCA1, a tumor suppressor protein mutated in hereditary breast and ovarian cancers (Venkitaraman 2002), is another candidate hTERT repressor acting through regulation of c-Myc. The effect of BRCA1 seems to involve a functional modification of the c-Myc protein rather than its downregulation (Figure 1). Li et al. (2002) found that a protein complex consisting of BRCA1, c-Myc and Nmi (originally identified as N-Myc-interacting protein) was responsible for the BRCA1-mediated repression of hTERT promoter in breast cancer cells. A recent report (Xiong et al. 2003) provided additional data showing that the overexpression of BRCA1 in breast cancer cells inhibits the transactivation function of the c-Myc protein, represses hTERT transcription, and down-regulates telomerase activity. While an siRNA against BRCA1 could block the binding of exogenous BRCA1 to a transfected hTERT promoter and the effects of exogenous BRCA1 on the activity of this promoter, further data on the regulation of the endogenous hTERT promoter by the endogenous BRCA1 protein are required to conclude that BRCA1 plays a physiological role in repressing hTERT expression in normal breast epithelial cells. Another c-Myc-related mechanism of hTERT transcriptional repression is the competition between Mad1 and c-Myc for their association with binding partner Max and/or their binding to E-box sequences. Mad1, a c-Myc antagonist, can repress hTERT transcription by competing and excluding c-Myc from the hTERT promoter, as well as by functioning as an active transcriptional repressor (Günes et al. 2000; Xu et al. 2001)(Figure 1).

The role of p53, a tumor suppressor most frequently mutated in various types of human cancers, in hTERT transcription is still controversial. Overexpression of the wild-type p53 in cervical cancer, Burkitt lymphoma and breast cancer cells led to repression of hTERT promoter activity (Kanaya et al. 2000; Xu et al. 2000). This repression seems to be mediated by an inhibitory interaction of overexpressed p53 with Sp1, a ubiquitous transcription factor essential to hTERT promoter activity (Figure 1). In support of a p53-mediated repression of hTERT transcription is the finding

that the loss of the endogenous p53 function led to the induction of hTERT expression and telomerase activity in an experimental cell system designed to study the immortalization and transformation of mammary epithelial cells (Stampfer et al. 2003). On the other hand, in an hTERT-negative osteosarcoma cell line expressing wild-type p53 (U2OS), RNAi inhibition of p53 function failed to induce hTERT expression. No difference in hTERT expression was observed in a pair of colon cancer cell lines (HCT116) that were isogenic except for p53 status (wild-type vs. homozygously knocked-out) (Lin and Elledge 2003). The apparent discrepancy between these above-mentioned studies might be explained by cell-type specificity, the use of different experimental conditions, and/or the status of other cellular factors controlling p53 function. Whether the hTERT repression is one of p53's tumor suppressor functions awaits accumulation of further experimental data. Recently, Lin and Elledge (2003) developed a retroviral mutagenesis-based genetic screen for the identification of hTERT transcriptional repressors. They not only confirmed that Mad1 and the TGF- β signaling pathway (described above) could repress hTERT transcription but they also identified Menin, a tumor suppressor protein mutated in familial multiple endocrine neoplasia type 1 (Guo and Sawicki 2001), as a novel candidate hTERT repressor. Using biologically relevant cell systems (e.g., in endocrine tumor cells and their normal counterparts for Menin), both Menin and tissue-specific tumor suppressor WT1 (Wilms' tumor 1; Oh et al. 1999) should further be characterized for their ability to act as hTERT repressor.

Investigation of endogenous hTERT repressors by chromosome transfer

While several known tumor suppressor proteins have been under investigation as endogenous hTERT repressors, we and others have used an experimental system based on microcell-mediated chromosome transfer (MMCT) to identify endogenous factors responsible for hTERT repression in normal cells (Oshimura and Barrett 1997; Ran and Pereira-Smith 2000). Because inactivation of tumor suppressor genes, including putative hTERT repressor genes, generally involves the loss or mutation of both alleles of the genes (so-called

'two hit'), introduction of a single copy of the genes under physiological expression levels (i.e., at the natural location on a normal chromosome) via MMCT is expected to restore their suppressor function in cancer cells. Several normal human chromosomes (i.e., chromosomes 3, 4, 6, 7, and 10) have been shown to repress the telomerase activity in some cancer cells (Ducrest et al. 2002; Horikawa and Barrett 2003; and references therein). In all the cases examined, repression of telomerase activity was associated with a repression of hTERT mRNA expression. These findings revealed endogenous mechanisms capable of repressing hTERT expression, which were restored by the transfer of these normal chromosomes. Indeed, an endogenous mechanism restored by an hTERT repressor gene on chromosome 3p21 acts on a specific DNA element within the hTERT promoter to repress the hTERT transcription in renal cancer cells (Horikawa et al. 2002). This DNA element was the canonical E-box sequence, where c-Myc binds and on which multiple hTERT regulatory mechanisms may also act. The difference in hTERT transcription between the renal cancer cells with and without the transferred chromosome 3 occurred in the absence of changes in the levels of the endogenous c-Myc or Mad1 proteins. Results from these investigations suggested that an unknown factor bound to the E-box element (either encoded or regulated by an hTERT repressor gene on chromosome 3p21) could not only inhibit c-Myc-mediated transactivation, but could also actively repress hTERT transcription (Horikawa et al. 2002 and unpublished observations) (Figure 1). This E-box-mediated repressive mechanism functions in various types of normal human cells including fibroblasts, mammary epithelial cells and prostate epithelial cells, while it is inactive in some, but not all, telomerase/hTERT-positive cancer cells (Horikawa et al. 2002). Cloning and characterization of the E-box-binding repressor would greatly facilitate our understanding of the endogenous mechanism by which normal human cells tightly repress hTERT transcription.

Cis-activation of hTERT transcription by viral genomes

A classic way by which tumor-associated viruses contribute to carcinogenesis is to activate cellular

genes with oncogenic potential through the integration within these genes of regulatory sequences (i.e., promoter or enhancer) from the viral genome (Makowski et al. 1984). This virus-mediated activation mechanism has also been shown to affect the hTERT locus (Figure 1). In hepatocellular carcinoma (HCC) cell line huH-4, the hepatitis B virus (HBV) genome is integrated within the hTERT promoter region, so that the hTERT mRNA is transcribed from both its endogenous promoter and the HBV promoter (Horikawa and Barrett 2001). The HBV enhancer sequence, inserted upstream of the hTERT promoter, was responsible for the activation in cis of the endogenous hTERT promoter. This finding, for the first time, showed that the structural alteration of a gene encoding a telomerase component resulted in the activation of telomerase in human cancers, adding the hTERT gene to the list of cellular targets that can be activated in cis by oncogenic viruses. Subsequently, Paterlini-Brechot et al. (2003) found two HCC cases (out of 22 HCC tissues examined) carrying a copy of the HBV genome integrated upstream of the hTERT promoter. Ferber et al. (2003) also reported one HCC case (out of 10 examined) and one HCC cell line (out of eight cell lines examined) that carried an hTERT locus containing an integrated copy of the HBV genome. Thus, the hTERT gene is a non-random integration site of, and possibly a target for cis-activation by, the viral genome in a fraction (possibly up to 10%) of HBV-positive HCCs. Three cases out of 109 HPV-positive cervical cancer tissues also had a copy of the HPV genome integrated upstream of the hTERT promoter (Ferber et al. 2003). Other human tumor-associated viruses deserve investigation for the possible integration into the hTERT gene. It would also be interesting to examine whether the integration-positive cases are associated with any specific clinical parameters, such as disease progression, prognosis, or tumor histology and grade.

Microenvironment and hTERT transcription

In addition to cellular and genetic changes intrinsic to the cancer cells themselves, the extracellular environment within which these cells develop (e.g., vascularization, oxygen concentration, glucose levels, immune and inflammatory cells, cytokines

and chemokines) can significantly influence the progression of human carcinogenesis (Henning et al. 2004). Hypoxia and the hypoxia-inducible factor (HIF), a transcriptional regulator, are known to play a key regulatory role in various aspects of tumor progression, including angiogenesis, glucose metabolism, cell proliferation and apoptosis (Acker and Plate 2002). Yatabe et al. (2004) reported that hypoxia increased telomerase activity in a uterine cervical cancer cell line through an HIF-1-mediated activation of hTERT transcription. The HIF-1 binding sites within the hTERT promoter overlapped with the E-box sequences, and HIF-1 seemed to compete with c-Myc for hTERT promoter binding. Under hypoxic conditions, HIF-1, rather than c-Myc, was associated with the hTERT promoter *in vivo*, where it functioned as the transcriptional activator (Yatabe et al. 2004). These findings suggest a possible mechanism by which tumor hypoxia and HIF-1 contribute to tumor progression *in vivo*. Koshiji et al. (2004) has revealed that HIF-1 is able to functionally counteract c-Myc in the transcriptional regulation of c-Myc-regulated genes (including p21^{CIP1}, BRCA1 and hTERT) in colon cancer cell line HCT116. Like other c-Myc-activated genes and in marked contrast with the results of Yatabe et al. (2004), the hTERT gene was repressed by both hypoxia and the stable expression of exogenous HIF-1. The biological significance of hypoxia-mediated hTERT repression remains to be determined, but transient genomic instability and dysfunctional telomeres associated with telomerase repression may play a key role at certain stages of carcinogenesis. Interestingly, the inhibition of the transcriptional function of c-Myc by HIF-1 does not require the C-terminal transactivation domain of the HIF-1 α subunit (Koshiji et al. 2004), a domain required for the recruitment by HIF-1 of transcriptional co-activator p300/CBP. It is likely that the activation and repression of hTERT transcription represent distinct functions of HIF-1. It would be interesting to examine whether the apparent discrepancy between the above-mentioned studies is explained by cell type differences in the response of hTERT to hypoxia or by variation in the status of other factors affecting hTERT transcription. Recent studies show that hTERT transcription and telomerase activity may be regulated by cytokines and growth factors that reflect local or systemic *in vivo* condi-

tions, such as interferon- γ , interleukin 6 and insulin-like growth factor 1 (Akiyama et al. 2002; Lee et al. 2003). Investigation of a link between the hTERT expression and tumor microenvironment is an important area of future research.

Conclusion and perspectives

Multiple mechanisms exist that control hTERT transcription, including its activation by cellular and viral oncogenic factors and its repression by tumor suppressor proteins and pathways (Figure 1 summarizes the major mechanisms described in this article). We previously proposed that an hTERT transcriptional regulator with biological relevance to the physiology of normal cells and/or pathology of cancer cells should: (1) exhibit its activity at physiological levels of expression; (2) act on the promoter or other regulatory regions of the endogenous hTERT gene; and (3) show alterations in the factor itself or its regulatory pathway during transition from normal to cancer cells (Horikawa and Barrett 2003). For hTERT activators such as the c-Myc and Ets families of transcription factors, data are accumulating that meet these criteria. In the case of the candidate hTERT repressors, further biochemical and functional analyses focused on the endogenous hTERT gene and the endogenously expressed proteins will be required. The MMCT-based approach to identify endogenous hTERT repressors is also an important area of research.

The activators and repressors of hTERT transcription may also function through modification of the chromatin structure of the hTERT gene locus. The acetylation status of the nucleosomal histones closely correlates with hTERT transcription (Xu et al. 2001; Hou et al. 2002). It is interesting to note that the protein TRRAP, which represents a component of a SAGA complex containing histone acetyltransferase hGCN5, interacts with c-Myc and is required for the activation of hTERT transcription by c-Myc (Nikiforov et al. 2002). The chromatin structure of the hTERT locus might represent the common endpoint of multiple regulatory mechanisms, and the characterization of its regulation may lead to a unifying model of hTERT transcriptional regulation from currently fragmentary findings pertaining to individual factors and pathways. Other

important issues to be addressed include the role of promoter DNA methylation in hTERT transcription (Devereux et al. 1999; Guilleret and Benhattar 2003) and a possible importance of other regulatory sequences located outside of the promoter (Leem et al. 2002; Wang et al. 2003). Future research in this field is expected to provide the basis for novel telomerase-based strategies for anti-cancer therapy.

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