Human telomerase reverse transcriptase (hTERT) transcription requires Sp1/Sp3 binding to the promoter and a permissive chromatin environment*

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*Running Head: hTERT regulation by Sp1 family transcription factors

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Background: Regulation of telomerase gene (hTERT) by Sp1 family proteins remains contradictory in previous studies.

Results: In chromatinized BAC reporters, Sp1/Sp3 binding to the promoter was essential for hTERT transcription, but did not affect its repression.

Conclusion: Chromatin remodeling, not Sp1 binding to the promoter, was a limiting step in hTERT transcription.

Significance: Chromatinized reporters are better models for studying telomerase regulation.

ABSTRACT

The transcription of human telomerase gene hTERT is regulated by transcription factors (TFs), including Sp1 family proteins, and its chromatin environment. To understand its regulation in a relevant chromatin context, we employed BAC reporters containing 160-kb of human genomic sequence containing the hTERT gene. Upon chromosomal integration, the BACs recapitulated endogenous hTERT expression, contrary to transient reporters. Sp1/Sp3 expression did not correlate with hTERT promoter activity and these TFs bound to the hTERT promoters in both telomerase-positive and -negative cells. Mutation of the proximal GC-box resulted in a dramatic decrease of hTERT promoter activity and mutations of all five GC-boxes eliminated its transcriptional activity. Neither mutations of GC-boxes nor knockdown of endogenous Sp1 impacted promoter binding by other TFs, including E-box binding proteins, and histone acetylation and trimethylation of histone H3K9 at the hTERT promoter in telomerase-positive and -negative cells. The result indicated that promoter binding by Sp1/Sp3 was essential, but not a limiting step, for hTERT transcription. hTERT transcription required a permissive chromatin environment. Importantly, our data also revealed different functions of GC-boxes and E-boxes in hTERT regulation: while GC-boxes were essential for promoter activity, factors bound to the E-boxes functioned to de-repress hTERT promoter.
successive cell divisions, leading to replicative senescence or cell death and functioning as a biological aging clock in somatic cells (6).

Telomerase regulation plays important roles in human aging and cancer. While TERC and other telomerase-associated proteins are ubiquitously expressed (7), hTERT is the limiting subunit of telomerase in many cells. Studies have shown that hTERT transcription is the primary step of telomerase regulation (8). The regulation of hTERT transcription requires binding of sequence-specific transcription factors (TFs), such as those of Sp1, c-Myc, USF, and E2F families, to the hTERT promoter (9-13). However, most of these TFs are widely expressed and as such their expression does not account for hTERT regulation during cell differentiation and tumorigenesis. Indeed, epigenetic mechanisms and chromatin environment also play critical roles in hTERT regulation (14). Our data showed that the hTERT gene was embedded in a condensed heterochromatin-like domain in many somatic cells (15). Inhibition of histone deacetylases (HDACs) led to an opening of this domain and activated hTERT transcription (14). It remains to be determined how TFs, such as Sp1, bind to the hTERT promoter in the context of this repressive chromatin environment (16).

Sp1 is a member of the Specificity Protein/Krüppel-like Factor (SP/KLF) TF family, which also includes Sp2, Sp3, and Sp4 (17). Sp1 and Sp3 are widely expressed, but the expression of Sp2 and Sp4 is restricted to specific tissues. Sp1 family TFs bind to consensus sequence GGGCGGGGG, known as GC-boxes. While Sp1 and Sp3 proteins are known to regulate transcription both positively and negatively, cumulative evidence also suggests that the chromatin context is an important factor in the differential loading of Sp1 and Sp3 to promoters (18).

The hTERT core promoter lacks a TATA box but contains an array of five GC boxes surrounded by two E-boxes, in addition to an initiator element (9,19). This arrangement is reminiscent of the TATA-less promoters found in many “housekeeping” genes (20). GC boxes are essential for promoter activity in many TATA-less promoters. However, studies on the role of Sp1 family proteins in hTERT regulation have yielded conflicting results thus far. Some reports indicated that Sp1 and GC-boxes played a positive role in hTERT transcription, because mutations of GC boxes reduced hTERT promoter activity (21). Conversely, other studies concluded that Sp1 family proteins and their binding sites were critical for the repression of hTERT promoter (22,23). Such puzzling results might have resulted from the fact that most of these investigations were based on transient transfection of small plasmid reporters, because previous studies demonstrated the limitation of transient assays in deciphering the function of Sp1/Sp3 factors and their binding sites in promoters (24).

To understand the roles of Sp1 family TFs in hTERT regulation, we generated mutations of GC-boxes at the hTERT promoter in a bacterial artificial chromosome (BAC) reporter, H(wt), containing 160-kb of human genomic DNA encompassing the consecutive CRR9 (also called CLPTM1L gene), hTERT, and Xtrp2 (or SLC6A18) loci. H(wt) and its mutant derivatives were then integrated at an acceptor site in telomerase-positive 3C167b (Tel') and -negative GM847 (Tel') cells, via Cre recombinase-mediated BAC targeting (RMBT) technique (25). Therefore, for the first time, the functions of GC-boxes and Sp1/Sp3 binding to these sites were examined in a relevant genomic and chromatin context. Our data indicated that the binding of Sp1 and Sp3 proteins to their cognate recognition sites were essential for the hTERT transcription in Tel' cells. Interestingly, Sp1 and Sp3 bound to the hTERT promoter in both Tel' and Tel'' cells, indicating that the binding of Sp1/Sp3 TFs were required but not sufficient for hTERT transcription and hTERT activation required a permissive chromatin environment. Furthermore, in this chromatinized reporter system, mutations of GC-boxes did not affect the states of histone acetylation and trimethylation of histone H3K9 at the hTERT promoter or binding of other TFs to the promoter. The residual activities of mutant promoters were induced proportionally as H(wt) upon inhibition of HDACs by trichostatin A (TSA), indicating that the binding of Sp1/Sp3 to the hTERT promoter had no significant impact on the repressive states of hTERT promoter. This was in sharp contrast to the proximal E-box at the hTERT promoter: its binding by TFs de-repressed the hTERT promoter.
EXPERIMENTAL PROCEDURES

BACs, plasmids, and transfection – hTERT promoter reporter plasmids were obtained from Dr. Horikawa (22). pBT-255 contained the 295-bp hTERT promoter fragment (-255 to +40nt, relative to the transcription start site, TSS, and directly upstream of the hTERT initiation codon) in pGL3-Basic vector (Promega, WI). pBT-255gc, or hM12 (22), contained a point mutation that eliminated the proximal GC-box, -31nt relative to TSS (Figure 1A). pLXSN-Sp1 was constructed by cloning a human Sp1 cDNA into retroviral vector pLXSN. In transient transfection assays with small plasmids, reporter and control plasmids were transfected into cells in triplicates using Lipofectamine 2000 (Life Technologies, NY) and luciferase activities were measured 48 hours later. Firefly luciferase (Fluc) activities were normalized to Renilla luciferase (Rluc) activities from co-transfected pRL-SV40 (Promega, WI).

BAC reporter constructs 117B23-cFtRvSVP, also named H(wt), and H(EboxD), which contained a mutation at the downstream E-box, were reported earlier (26). H(gc) and H(gc5) contained point mutations at the proximal GC-box (-31nt) and all five GC-boxes (-132, -112, -78, -58, and -31nt) at the hTERT promoter, respectively (Figure 1A). The mutations in the BAC reporters were generated using our two-step BAC recombinaseering method (27). In transient experiments, BACs were transfected into cells using FuGENE® HD (Roche, NJ) and luciferase assays were performed in 48 hours. Chromatin Immunoprecipitation (ChIP) – ChIP was performed as we reported previously (26). Antibodies used in ChIP experiments are Sp1 & Sp3 (07-645 & 07-107, EMD Millipore, Billerica, MA) and E2F1-3 (sc-193, sc-633, & sc878, Santa Cruz Biotechnology, CA). All other antibodies were described previously (26). Primer sequences used for CHIP are listed in Table 1.

Gene expression analyses – Totally RNAs were extracted from culture cells and reverse transcription and quantitative PCR (qPCR) were performed as described previously (28). Primers are shown in Table 1 or published previously (26). Western analyses were performed as described in (26).

RESULTS

The core hTERT promoter contains a set of five GC boxes, which have been reported to play critical roles in hTERT regulation, either activation or repression of the hTERT promoter. Our previous data showed that the hTERT gene was embedded in a nuclease-resistant chromatin domain in human somatic cells (15). The hTERT promoter was repressed in both telomerase-positive and -negative cells, and the repression was especially stringent in telomerase-negative cells. We set out to determine the roles of Sp1 family TFs in the regulation of hTERT transcription in this repressive chromatin environment. First, a set of human fibroblast cells was selected for this study (14): telomerase-positive 3C167b and GM639, telomerase-negative ALT lines 3C166a and GM847, as well as NHFs. Their endogenous hTERT mRNA expression was validated by qRT-PCR analysis (Figure 1B). While Sp1 and Sp3 were expressed in all the cells, their protein levels did not correlate with hTERT mRNA levels (Figure 1C). Thus, the expression of Sp1 and Sp3 proteins did not account for the differential hTERT expression in these cells.
In most previous studies, small plasmid reporters were used to understand the hTERT promoter functions. Thus, we first used pBT-255, a firefly luciferase reporter plasmid containing a 295-bp hTERT promoter fragment (29), and its derivative pBT-255gc containing point mutations at the proximal GC-box (22). This promoter fragment contained all the TF binding sites shown in Figure 1A, including five GC-boxes, two E-boxes, and three E2F sites. As shown in Figure 2A, mutation of the proximal GC-box resulted in a 30% decrease in promoter activity in both telomerase-positive 3C167b and -negative GM847 (Tel-) cells, designated hereinafter as Tel+ and Tel- cells, respectively. In both cells, overexpression of Sp1 protein led to an approximately 2-fold increase of the hTERT promoter activity (Figures 2B&C). Conversely, knockdown of Sp1 expression using lentiviral shRNAs resulted in a significant reduction of luciferase activity of pBT-255 (Figure 2D). Therefore, in this transient plasmid reporter system, Sp1 factor and the GC-boxes mediated the activation of the hTERT promoter.

To determine the potential roles of distal regulatory elements in hTERT regulation, we introduced H(wt), a BAC reporter which contains a 160-kb human genomic DNA encompassing the CRR9, hTERT, and Xtrp2 genes, as shown in Figure 1A. A Renilla (Rluc) and a Firefly luciferase (Fluc) cassette were inserted to the initiation codons of hTERT and CRR9 genes, respectively. Because CRR9 was ubiquitously expressed in all cells and tissues examined (30), Fluc was used as an internal control and the ratio of Rluc to Fluc activities was a direct measurement of hTERT promoter activity. As shown in Figure 3A, upon transient transfection of H(wt), Rluc/Fluc ratios varied in different cell lines, but did not correlate with endogenous hTERT expression (Figure 2B). Sp1 overexpression resulted in 40% to one-fold increases in the hTERT promoter activity in H(wt) (Figure 3B), consistent with an activator role of the Sp1 protein.

To test the functions of Sp1 in the context of BAC reporters, mutations were introduced into GC-boxes in H(wt). H(gc) contained a point mutation in the proximal GC-box, whereas all five GC-boxes in the promoter region were mutated in H(gc5) (Figure 1A). In both Tel+ and Tel- cells, hTERT promoter activity was reduced by 20-30% in H(gc) and by 40-50% in H(gc5) in transient transfection of BAC reporters (Figure 3C). The impact of GC-box mutations on hTERT promoter in the BAC reporter was similar to that in plasmid reporter pBT-255. Overexpression of Sp1 activated the hTERT promoter in wildtype H(wt) by about two-fold and its effect on H(gc) and H(gc5) was greatly diminished (Figure 3D), indicating that Sp1 activated hTERT promoter primarily via these GC-boxes.

Chromatin is an essential component of hTERT regulation (16). To determine the roles of GC-boxes and their binding proteins in the hTERT regulation in a relevant chromatin context, we used our previously reported RMBT method to integrate single-copy BAC reporters into chromosomal acceptor sites in Tel+ and Tel- cells (Figure 4A) (25). As we previously reported, the hTERT promoter in H(wt) was over 20-fold stronger in Tel+ cells than in Tel- cells (Figure 4B). HDAC inhibition by TSA dramatically induced hTERT promoter activity in both cells, indicating that the chromatinized H(wt) recapitulated the repression of endogenous hTERT locus.

To determine the binding of Sp1 protein to the hTERT promoters, ChIP experiments were performed. As shown in Figure 4C, while the association of E-box-binding proteins Max and USF1/2 with the endogenous and transgenic hTERT promoters was higher in Tel+ cells than in Tel- cells, Sp1 bound to the hTERT promoters similarly in these two cells. As a control, trimethylation of H3K4 was significantly higher in Tel+ cells than in Tel- cells, consistent with hTERT transcription in Tel+ cells. Thus, Sp1 binding to hTERT promoter occurred in both Tel+ and Tel- cells and did not correlate with hTERT activation. However, when Sp1 was overexpressed, the chromatinized hTERT promoters were up-regulated by about 2-fold in both Tel+ and Tel- cells (Figure 4D).

To decipher the role of GC-boxes in a relevant chromatin context, H(gc) and H(gc5) were inserted into the same chromosomal acceptor sites as H(wt) in Tel+ and Tel- cells via RMBT (Figures 1A & 4A). As shown in Figure 5A, mutation of the proximal GC-box in H(gc) resulted in over 80% loss of hTERT promoter activity, and
mutations of all five GC-boxes virtually abolished promoter function. When HDACs were inhibited by TSA, Rluc expression from H(gc) was proportionally induced, compared to those from H(wt) in Tel+ and Tel- cells. TSA failed to induce Rluc expression from H(gc5), consistent with that the hTERT promoter function was abolished in this reporter. This was in sharp contrast to H(EboxD), in which the downstream E-box was mutated. The hTERT promoter activity in H(EboxD) was reduced by 5-fold compared to that of H(wt). However, upon TSA treatment, the mutant promoter was induced to a level similar to those of wildtype promoter in H(wt) in both Tel+ and Tel- cells. This result suggested that GC-boxes and E-boxes played different roles in hTERT regulation. Whereas proteins that bind to E-boxes might recruit histone acetyltransferases (HATs) and de-repress the hTERT promoter, the binding of Sp1 family TFs to GC-boxes was likely essential for its promoter function.

ChIP experiments revealed that the binding of Sp1 and Sp3 to the transgenic promoter was significantly reduced in mutant BACs H(gc) and H(gc5) (Figure 5B). As a control, Sp1/Sp3 binding to the endogenous hTERT promoter was unaffected in the same cells. Mutations of the GC-boxes were accompanied by a reduction of di- and tri-methylation of histone H3 lysine 4 (H3K4me2 and H3K4me3) (Figure 5C), consistent with reduced transcriptional activities of the mutant promoters. However, the recruitment of E-box factors, Max and USF1/2, to the mutant promoters was not affected. Therefore, these results indicated that the binding of Sp1 family proteins to GC-boxes was critical for the promoter activity, but did not affect TF binding to neighboring E-boxes, which activated hTERT transcription by de-repressing the promoter (26). Consistently, these GC-box mutations did not affect acetylation status of histones H3 and H4 (H3Ac and H4Ac), trimethylation of histone H3 lysine 9 (H3K9me3), and the association of histone H1 at the hTERT promoters (Figure 5C), supporting the notion that Sp1/Sp3 binding did not influence the repressive chromatin state of hTERT promoter.

To further determine the roles of Sp1 family TFs in regulating hTERT promoter activity, the endogenous expression of Sp1 and Sp3 was knocked down by lentiviral shRNAs. As shown in Figure 6A, two shRNAs against Sp1, shSp1a and shSp1b, reduced its protein level by 90% and 70% and two shRNAs, shSp3a and shSp3b, inhibited Sp3 expression by 70% and 30%, respectively. KD of either Sp1 or Sp3 resulted in over 50% decreases of both endogenous hTERT mRNA levels (Figure 6B) and transcription from the transgenic hTERT promoter in chromatinized H(wt) (Figure 6C). Co-transductions of cells with lentiviral shRNAs against Sp1 and Sp3 further reduced the expression from endogenous and transgenic hTERT promoters, indicating that both Sp1 and Sp3 contributed to hTERT transcriptional activation.

Finally, ChIP experiments were performed to determine how Sp1 KD affected the chromatin structure of the hTERT promoters. Sp1 KD reduced the association of Sp1 with both endogenous and transgenic hTERT promoters, but had no overall effects on the binding of other TFs, such as E-box binding factors and E2F family proteins (Figure 7A). Moreover, H3Ac, H4Ac, H3K4me2, and H3K4me3 were not consistently affected by Sp1 KD (Figure 7B).

**DISCUSSION**

Sp1 family proteins, Sp1 and Sp3, are near ubiquitous TFs (17). They can function as activators or repressors depending on their binding sites and interacting cofactors (31). Here, we studied the roles of these TFs at the hTERT promoter in the context of a chromatinized transgenic BAC reporter. Our data showed that, although the expression of Sp1 and Sp3 did not correlate with hTERT transcription, they both contributed to hTERT transcriptional activation and their binding sites, the five GC-boxes, were crucial for the hTERT promoter function. While this conclusion was not a surprise given that some of previous publications using transient plasmid reporters also showed that Sp1 family TFs and the GC-boxes were involved in hTERT activation, our current study using chromatinized BAC reporters demonstrated that these factors were not involved in hTERT repression, in contrast to several earlier reports (22,23). Moreover, the repressive states of hTERT promoter in Tel+ and Tel- cells did not seem to affect Sp1 binding to the promoter, nor did Sp1/Sp3 binding to GC-boxes significantly change the states of histone acetylation and
trimethylation of H3K9, a repressive chromatin mark, at the hTERT promoter. In Tel+ cells, the hTERT promoter was silenced while Sp1 bound to the promoter, indicating the limitation for hTERT transcription occurred at steps following Sp1 binding.

There were several advantages using chromatinized BAC reporters to study hTERT regulation. First, the expression from transgenic hTERT promoter in chromosomally integrated H(wt) recapitulated endogenous hTERT transcription in host cells (25), whereas the hTERT promoter in small plasmid reporters or even the transient BAC reporter H(wt) bore no resemblance to the host gene, indicating that some of regulatory components were missing in transient reporter systems. It was likely that the repressive chromatin environment crucial for hTERT regulation was absent in transiently transfected DNA constructs (16). Second, the expression of luciferase reporter in chromatinized H(wt) depended much more on the GC-boxes at the hTERT promoter than those of transiently transfected hTERT reporters. Mutation of the proximal GC-box reduced transcription from the hTERT promoter on pBT-255 by 40-60% (Figure 2A) and that from the transiently transfected H(wt) by 20-30% (Figure 3C). In contrast, the same mutation reduced hTERT promoter in chromatinized H(gc) by 7-fold (Figure 5A). Mutation of all five GC-boxes in H(gc5) decreased hTERT promoter activity in Tel+ cells by 300-fold and effectively eliminated hTERT promoter activity even in the presence of HDAC inhibitor TSA in both Tel+ and Tel- cells. The result indicated that it was easier for RNA Pol II and general transcription factors to be loaded onto the transiently transfected DNA constructs than onto chromatinized reporters or endogenous genes, likely due to the lack of an appropriately assembled nucleosomal array in these newly transfected DNA (32,33). In chromatinized BAC reporters, transcriptional initiation depended strictly on the hTERT promoter, making them a more reliable model for studying hTERT regulation.

Repressive chromatin environment, signified by histone deacetylation and other histone modifications, such as H3K9me3, was a key component of hTERT regulation (14). In a previous study, we showed that the hTERT locus was embedded in a condensed heterochromatin-like domain in many somatic cells (15). This domain was present in both 3C167b (Tel+) and GM847 (Tel-) cells used in this study. How hTERT transcription occurs in such a repressive chromatin is currently unknown and a focus of our research. hTERT transcription in Tel+ cells correlated with the appearance of a major DNase I hypersensitive site (DHS) at the hTERT promoter (14,34). Because Sp1 binding induced an asymmetric bend in DNA (35), we previously speculated that Sp1 binding might have caused DNA bending and nucleosome sliding, resulting in the formation of a DHS (14). In this study, we showed that Sp1 bound to the hTERT promoter not only in Tel+ cells, but also in Tel- cells without this DHS (14). As such, Sp1 binding was insufficient to initiate hTERT transcription (Figure 8, top). Because E-box-binding proteins, Myc family proteins and USF1/2, were more enriched on hTERT promoter in Tel+ cells than in Tel- cells, these factors might be directly involved in local de-repression/activation of the hTERT promoter in Tel+ cells. However, because these TFs were also abundant in Tel+ cells (26), their binding to the hTERT promoter in Tel+ cells was likely a result of chromatin remodeling, possibly the establishment of DHS at hTERT promoter (Figure 8, middle). The binding of these TFs may further recruit additional histone modifying factors and remodel the +1 nucleosome immediately downstream of the hTERT TSS, leading to its transcription in a permissive chromatin environment (Figure 8, bottom). The current data, together with our earlier study on temporal repression of the hTERT gene during HL60 cell differentiation (36), suggested that local chromatin remodeling to create a nucleosome-free region (i.e., DHS) at the hTERT promoter, instead of TF binding, was a limiting step in hTERT transcription. This model is consistent with the data that transiently transfected hTERT promoters were active in both Tel+ and Tel- cells. Identification of the events that trigger chromatin remodeling is likely a key to understand hTERT transcriptional activation.

Another new finding in the current study was that Sp1 family TFs and E-box binding proteins played different roles in hTERT transcription.
Mutation of the downstream E-box significantly reduced hTERT promoter activity, but this reduction disappeared upon inhibition of HDACs by TSA. Therefore, it is likely that E-box binding proteins recruit HATs to the promoter, de-repressing the hTERT promoter and thereby activating hTERT transcription. On the other hand, binding of Sp1 family TFs to GC-boxes was essential for hTERT promoter function. Mutations of GC boxes or KD of Sp1 protein reduced promoter activity, but had no effect on the histone acetylation and the recruitment of other TFs. The functions of Sp1 at hTERT promoter might include recruit Pol II containing pre-initiation complex, or PIC. We attempted to determine the effect of Sp1 KD on Pol II recruitment, but were unable to obtain conclusive results due to low Pol II ChIP signals (Figures 5B & 7A). This is not surprising because hTERT promoter is under strong HDAC-mediated repression in Tel+ cells and a previous report showed that hTERT gene is transcribed at a low level even in cancer cells, which contained no more than a few copies of mRNA per cell (14,37).

Although induction of hTERT expression by TSA suggested that repression required histone deacetylation, the possibility of its indirect effects on the hTERT promoter could not be ignored. However, we previously showed that the TSA-induced hTERT expression and alteration of chromatin configuration were not blocked by the protein synthesis inhibitor cycloheximide (14,37), indicating that TSA-induced hTERT transcription did not require new protein synthesis and thus was likely a result of HDAC inhibition at the hTERT promoter. In the current study, we further tested several potential hTERT activators, c-Myc/Max, Sp1, and p53. As shown in Figure 9, only the c-Myc mRNA level was elevated in Tel+ cells upon TSA treatment, but its protein level was not increased in these cells. Therefore, the regulation of these TFs could not account for the dramatic activation of hTERT transcription following HDAC inhibition by TSA.

It was reported that Sp1 and Sp3 were subjected to a variety of post-translational modifications, including phosphorylation, ubiquitination, acetylation, sumylation, glycosylation, and poly(ADP-ribosyl)ation (reviewed in (38)). While some of these modifications may impact their DNA-binding activities, other modifications may affect the abilities of Sp1 family TFs to interact with other TFs and chromatin factors. It remains to be determined whether Sp1 and Sp3 proteins are subjected to differential modifications in Tel+ and Tel− cells.

In this study, we used immortal Tel+ and Tel− cell lines that were derived from SV40 large T antigen-transformed human diploid fibroblasts (14). First, these cell lines were chosen for the current study because the RMBT method involved two rounds of clonal expansion of the host cells, the insertion of a retroviral acceptor site into a host cell chromosome and Cre-mediated integration of the BAC reporters. This precluded the use of most primary human cells due to their limited lifespan in culture. Second, since Tel+ and Tel− cells are both SV40 transformed IMR90 cells, their genetic backgrounds are more related than most cancer cell lines and especially suited for studying hTERT activation during immortalization of human fibroblasts following M2 crisis (39). One caveat is that inactivation of p53 and pRb pathways by T antigens alters cell cycle control and consequently impacts on hTERT regulation in these cells. However, mutations of these two tumor suppressor pathways occur in majority of cancers, making these cells relevant models for studying telomerase activation during tumorigenesis. In addition, the chromatin state of the hTERT promoter is likely very complex. This study examined only a limited set of histone and epigenetic modifications. Further analyses of additional epigenetic marks, such H3K27 methylation and DNA methylation, may shed more light on how the hTERT promoter is regulated by Sp1 TFs in these cells.

In summary, we focused on the role of Sp1 family TFs in hTERT regulation in a relevant genomic and chromatin context in this study. Our data showed that the binding of these TFs to the GC-boxes were essential, but not sufficient, for hTERT activation. hTERT transcription occurred only in a permissive chromatin environment and Sp1 family TFs played no roles in this repression.
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REFERENCES

1. Morin, G. B. (1989) The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 59, 521-529

2. Shay, J. W., and Wright, W. E. (2010) Telomeres and telomerase in normal and cancer stem cells. *FEBS Lett* 584, 3819-3825

3. Venteicher, A. S., Abreu, E. B., Meng, Z., McCann, K. E., Terns, R. M., Veenstra, T. D., Terns, M. P., and Artandi, S. E. (2009) A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. *Science* 323, 644-648

4. Cohen, S. B., Graham, M. E., Lovrecz, G. O., Bache, N., Robinson, P. J., and Reddel, R. R. (2007) Protein composition of catalytically active human telomerase from immortal cells. *Science* 315, 1850-1853

5. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. (1994) Specific association of human telomerase activity with immortal cells and cancer. *Science* 266, 2011-2015

6. de Lange, T. (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 19, 2100-2110

7. Blasco, M. A., Rizen, M., Greider, C. W., and Hanahan, D. (1996) Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. *Nature Genetics* 12, 200-204

8. Aisner, D. L., Wright, W. E., and Shay, J. W. (2002) Telomerase regulation: not just flipping the switch. *Curr Opin Genet Dev* 12, 80-85.

9. Takakura, M., Kyo, S., Kanaya, T., Hirano, H., Takeda, J., Yutsudo, M., and Inoue, M. (1999) Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. *Cancer Res* 59, 551-557

10. Wu, K. J., Grandori, C., Amacker, M., Simon-Vermot, N., Polack, A., Lingner, J., and Dalla-Favera, R. (1999) Direct activation of TERT transcription by c-MYC. *Nature Genetics* 21, 220-224

11. Kyo, S., Takakura, M., Taira, T., Kanaya, T., Itoh, H., Yutsudo, M., Ariga, H., and Inoue, M. (2000) Spl1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nuc Acids Res* 28, 669-677

12. Goueli, B. S., and Janknecht, R. (2003) Regulation of telomerase reverse transcriptase gene activity by upstream stimulatory factor. *Oncogene* 22, 8042-8047

13. Xu, D., Dwyer, J., Li, H., Duan, W., and Liu, J. P. (2008) Ets2 maintains hTERT gene expression and breast cancer cell proliferation by interacting with c-Myc. *J Biol Chem* 283, 23567-23580

14. Wang, S., and Zhu, J. (2003) Evidence for a relief of repression mechanism for activation of the human telomerase reverse transcriptase promoter. *J Biol Chem* 278, 18842-18850
15. Wang, S., and Zhu, J. (2004) The hTERT gene is embedded in a nuclease-resistant chromatin domain. *J Biol Chem* **279**, 55401-55410

16. Zhu, J., Zhao, Y., and Wang, S. (2010) Chromatin and epigenetic regulation of the telomerase reverse transcriptase gene. *Protein & Cell* **1**, 22-32

17. Suske, G., Bruford, E., and Philipsen, S. (2005) Mammalian SP/KLF transcription factors: bring in the family. *Genomics* **85**, 551-556

18. Hodny, Z., Li, R., Barath, P., and Nelson, B. D. (2000) Sp1 and chromatin environment are important contributors to the formation of repressive chromatin structures on the transfected human adenine nucleotide translocase-2 promoter. *Biochem J* **346 Pt 1**, 93-97.

19. Crowe, D. L., Nguyen, D. C., Tsang, K. J., and Kyo, S. (2001) E2F-1 represses transcription of the human telomerase reverse transcriptase gene. *Nuc Acids Res* **29**, 2789-2794

20. Azizkhan, J. C., Jensen, D. E., Pierce, A. J., and Wade, M. (1993) Transcription from TATA-less promoters: dihydrofolate reductase as a model. *Crit Rev Eukaryot Gene Expr* **3**, 229-254

21. Takakura, M., Kyo, S., Sowa, Y., Wang, Z., Yatabe, N., Maida, Y., Tanaka, M., and Inoue, M. (2001) Telomerase activation by histone deacetylase inhibitor in normal cells. *Nuc Acids Res* **29**, 3006-3011

22. Horikawa, I., Chiang, Y. J., Patterson, T., Feigenbaum, L., Leem, S. H., Michishita, E., Larionov, V., Hodes, R. J., and Barrett, J. C. (2005) Differential cis-regulation of human versus mouse TERT gene expression in vivo: identification of a human-specific repressive element. *Proc Natl Acad Sci. USA* **102**, 18437-18442

23. Won, J., Yim, J., and Kim, T. K. (2002) Sp1 and Sp3 Recruit Histone Deacetylase to Repress Transcription of Human Telomerase Reverse Transcriptase (hTERT) Promoter in Normal Human Somatic Cells. *J Biol Chem* **277**, 38230-38238.

24. Davie, J. R., He, S., Li, L., Sekhavat, A., Espino, P., Dro bic, B., Dunn, K. L., Sun, J. M., Chen, H. Y., Yu, J., Pritchard, S., and Wang, X. (2008) Nuclear organization and chromatin dynamics--Sp1, Sp3 and histone deacetylases. *Advances in enzyme regulation* **48**, 189-208

25. Wang, S., Zhao, Y., Leiby, M. A., and Zhu, J. (2009) Studying human telomerase gene transcription by a chromatinized reporter generated by recombinase-mediated targeting of a bacterial artificial chromosome. *Nuc Acids Res* **37**, e111

26. Zhao, Y., Cheng, D., Wang, S., and Zhu, J. (2014) Dual roles of c-Myc in the regulation of hTERT gene. *Nuc Acids Res* **42**, 10385-10398

27. Wang, S., Zhao, Y., Leiby, M., and Zhu, J. (2009) A new positive/negative selection scheme for precise BAC recombinering. *Mol Biotechnol* **42**, 110-116

28. Jia, W., Wang, S., Horner, J. W., Wang, N., Wang, H., Gunther, E. J., DePinho, R. A., and Zhu, J. (2011) A BAC transgenic reporter recapitulates in vivo regulation of human telomerase reverse transcriptase in development and tumorigenesis. *Faseb J* **25**, 979-989

29. Horikawa, I., Cable, P. L., Mazur, S. J., Appella, E., Afshari, C. A., and Barrett, J. C. (2002) Downstream E-Box-mediated Regulation of the Human Telomerase Reverse Transcriptase (hTERT) Gene Transcription: Evidence for an Endogenous Mechanism of Transcriptional Repression. *Mol Biol Cell* **13**, 2585-2597.

30. Wang, S., Robertson, G. P., and Zhu, J. (2004) A novel human homologue of Drosophila polycomblike gene is up-regulated in multiple cancers. *Gene* **343**, 69-78

31. Kaczynski, J., Cook, T., and Urrutia, R. (2003) Sp1- and Kruppel-like transcription factors. *Genome biology* **4**, 206

32. Hebbar, P. B., and Archer, T. K. (2008) Altered histone H1 stoichiometry and an absence of nucleosome positioning on transfected DNA. *J Biol Chem* **283**, 4595-4601
33. Jeong, S., and Stein, A. (1994) Micrococcal nuclease digestion of nuclei reveals extended nucleosome ladders having anomalous DNA lengths for chromatin assembled on non-replicating plasmids in transfected cells. *Nuc Acids Res* **22**, 370-375

34. Wang, S., Zhao, Y., Hu, C., and Zhu, J. (2009) Differential repression of human and mouse TERT genes during cell differentiation. *Nuc Acids Res* **37**, 2618-2629

35. Sjottem, E., Andersen, C., and Johansen, T. (1997) Structural and functional analyses of DNA bending induced by Sp1 family transcription factors. *Journal of Molecular Biology* **267**, 490-504

36. Wang, S., Hu, C., and Zhu, J. (2010) Distinct and Temporal Roles of Nucleosomal Remodeling and Histone Deacetylation in the Repression of the hTERT Gene. *Mol Biol Cell* **21**, 821-832

37. Ducrest, A. L., Amacker, M., Mathieu, Y. D., Cuthbert, A. P., Trott, D. A., Newbold, R. F., Nabholz, M., and Lingner, J. (2001) Regulation of human telomerase activity: repression by normal chromosome 3 abolishes nuclear telomerase reverse transcriptase transcripts but does not affect c-Myc activity. *Cancer Res* **61**, 7594-7602.

38. Wierstra, I. (2008) Sp1: emerging roles--beyond constitutive activation of TATA-less housekeeping genes. *Biochem Biophys Res Commun* **372**, 1-13

39. Zhu, J., Wang, H., Bishop, J. M., and Blackburn, E. H. (1999) Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. *Proc Natl Acad Sci. USA* **96**, 3723-3728
FOOTNOTES
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The abbreviation used: BAC, bacterial artificial chromosome; DHS, DNase I hypersensitive site; Fluc, firefly luciferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; hTERT, human telomerase reverse transcriptase; KD, knockdown; NHF, normal human fibroblast; Rluc, Renilla luciferase; RMBT, recombinase-mediated BAC targeting; Sp1, specificity protein 1; Tel+, telomerase-positive cells (3C167b); Tel-, telomerase-negative cells (GM847); TF, transcription factor; TSA, trichostatin A; TSS, transcription start site.

FIGURE LEGENDS

Figure 1. The regulation of hTERT gene and Sp1 expression. A. The hTERT gene and promoter mutations. Top portion is a 160-kb human genomic region containing the hTERT, CRR9, and Xtrp2 loci. Exons are designated as vertical bars and lines. Black portions of horizontal lines represent repetitive sequences. Horizontal arrows indicate directions of transcription. The 295-bp core promoter upstream of hTERT initiation codon is shown in the lower portion. BAC and plasmid constructs containing the mutations are listed on the right. B. hTERT mRNA expression in fibroblasts. mRNA levels were determined by qRT-PCR analyses and normalized to 18S ribosomal RNA. NHF, GM847, and 3C166a were telomerase-negative cells, whereas GM639 and 3C167b were telomerase-positive lines. C. Expression of Sp1 and Sp3 TFs. Protein expression was determined by Western analyses using antibodies as indicated on the left.

Figure 2. Regulation of transiently transfected hTERT promoter reporter. A. Activities of transiently transfected hTERT promoter. Plasmid reporters were transfected into Tel+ (3C167b) and Tel- (GM847) cells. B. Overexpression of Sp1 protein. Tel+ and Tel- cells were transfected with pLXSN-Sp1 or pLXSN vector only and harvested for Western analysis in two days. C. Regulation of transient hTERT plasmid reporters by Sp1 overexpression. hTERT reporters were cotransfected with either pLXSN-Sp1 or pLXSN vector into Tel+ (upper panel) and Tel- (lower panel) cells. D. Regulation of transient reporter pBT-255 by shRNAs against Sp1. pBT-255 and pLKO plasmids containing shSp1a, spSp1b, or a scrambled sequence (SCR) were cotransfected into Tel+ cells. In all transient reporter assays, Firefly luciferase activities were measured 48h post transfection and normalized to Renilla luciferase activities from cotransfected plasmid pRL-SV40.

Figure 3. The activity of hTERT promoter in transient BAC reporter H(wt). A. Transiently transfected BAC H(wt) in fibroblast lines. H(wt) was transfected into cells and luciferase activities were measured 48h later. hTERT promoter activity was measured as the ratio of Renilla luciferase (Rluc) from the hTERT promoter to Firefly luciferase (Fluc) from the CRR9 promoter. B. Regulation of the hTERT promoter in transiently transfected H(wt) by Sp1. Tel+ (3C167b) and Tel- (GM847) cells were cotransfected with H(wt) and either pLXSN-Sp1 or pLXSN vector only. Luciferase assay were performed 48h after transfection. C. Effects of GC-box mutations in H(wt). H(wt) and mutant BACs were transfected in to Tel+ and Tel- cells and luciferase activities were measured 2 days later. D. Activation of wildtype and mutant hTERT promoter in transient BAC reporters by Sp1. BACs were cotransfected with either pLXSN-Sp1 or pLXSN vector and luciferase activities were measured in 48h.

Figure 4. Chromatinized reporter H(wt). A. A schematic illustration of the RMBT strategy. Lox511 and loxP are represented by black and grey triangles, respectively. The acceptor locus is surrounded by a chicken β-globin insulator chS4 (‘Ins’ in an octagon) on each side. B. hTERT promoter activity in a chromosomal BAC reporter H(wt) in Tel+ and Tel- lines. Luciferase activities were measured in cells from 96-well plates treated without (-TSA) or with 250nM TSA (+TSA) for 24h. The hTERT promoter activities are shown as Rluc/Fluc. C. TF binding and histone modifications at the transgenic and endogenous hTERT promoters. Chromatin fragments from Tel+/H(wt) and Tel-/H(wt) cells were
precipitated using antibodies against TFs and H3K4me3, followed by qPCR analyses. Shown are precipitated fragments as percentages of input chromatin. Positions of PCR amplicons for ChIP analyses are shown below. 1, Up5k or 5-kb upstream of the hTERT promoter; 2, endogenous promoter; 3, transgenic promoter; 4, RlucO or Rluc ORF. Grey rectangles represent exons 1 and 2 of the hTERT gene. TSS, transcription start sites. D. Regulation of the chromatinized hTERT promoter by Sp1. Tel+/H(wt) and Tel-/H(wt) cells were transfected with pLXSN-Sp1 or pLXSN vector and luciferase activities were measured 2 days later.

Figure 5. Effects of GC-box mutations on chromosomal hTERT promoters. A. The activities of wildtype and mutant hTERT promoters. Tel+ and Tel- cells containing integrated H(wt), H(gc), H(gc5), and H(EboxD) were treated without (-TSA, upper chart) or with (+TSA, lower chart) 250nM TSA for 24 hours prior to harvesting. B & C. TF binding (B) and histone modifications (C) at the hTERT promoters in the wildtype and mutant BACs. Chromatin fragments from Tel+ cells containing BAC reporters were precipitated using antibodies against TFs (B) or specific histone modifications (C), followed by qPCR analyses. Shown are precipitated fragments as percentages of input chromatin. H3Ac and H4Ac refer to acetylated histone H3 and H4, respectively. H3K4me2 and H3K4me3 are di- and tri-methylated H3K4.

Figure 6. Effects of knockdown (KD) of Sp1 family proteins on hTERT regulation in in Tel+ cells. A. KD of Sp1 and Sp3 proteins by shRNAs. Tel+ cells were infected with lentiviral shRNAs against Sp1 or Sp3 for 4 days. Protein expression was determined by Western analyses using antibodies as indicated. B. Effects of Sp1/Sp3 KD on endogenous hTERT expression. Tel+ cells were infected with lentiviral shRNAs and selected with 1 µg/ml puromycin for 4 days. hTERT mRNA levels were determined by qRT-PCR and normalized to 18S rRNA. C. Effects of Sp1/Sp3 KD on chromatinized hTERT promoter. Tel+/H(wt) cells were infected with lentiviral shRNAs and luciferase activities were measured 3 & 4 days post infection. hTERT promoter activities were determined as Rluc/Fluc. Vec, lentiviral vector pLKO.1; SCR, scrambled shRNA.

Figure 7. The effects on chromatin structure by Sp1 KD. TF binding (A) and histone modifications (B) at the endogenous and transgenic hTERT promoters. Tel+/H(wt) cells were infected with lentiviruses and harvested for ChIP assays 4 days post infection.

Figure 8. A model of hTERT transcription. Genomic DNAs are represented by horizontal lines and hTERT exons are as shown gray rectangles. Nucleosomes are depicted as circles. Top, the hTERT promoter is repressed in Tel- cells. While the promoter is bound by Sp1 family TFs, it is not accessible to other TFs. Middle, dashed arrows indicate that the binding sites on the hTERT promoter are accessible to TFs in a permissive chromatin environment. Bottom, in Tel+ cells, the binding of E-box binding proteins and other TFs lead to further remodeling of the +1 nucleosome immediately downstream of TSS, indicated by the dashed oval. Transcription is indicated by the horizontal arrow on the active promoter.

Figure 9. Effects of potential transcriptional regulators by TSA treatment. A. Regulation of mRNA levels by TSA treatment. Tel+ (3C167b) and Tel- (GM847) cells were treated without or with 0.2 µM TSA for 24h. Total RNAs were harvested and analyzed by quantitative RT-PCR. The data were normalized to 18S rRNA. B. Effect of c-Myc protein levels by TSA treatment. Tel+ and Tel- cells were treated with 0.5µM TSA for 24h. Protein expression was determined by Western analyses.
Table 1. Sequences of PCR primers and small hairpin RNAs (5’→3’)

| qRT-PCR primers | Targets | Forward | Reverse |
|-----------------|---------|---------|---------|
|                 | hTERT   | GAACATGCGTGCACAACTTGTGG | TGCAGCAGGAGGATCTTGTAGATG |
|                 | 18sRNA  | TAGAGGGACACAGTGCGTTCT | CGCTGACCAGTCAGTG |
|                 | p53     | CAACAGACAGCTGCTCCT | CATCAAGCTCGGAACATCTC |

| ChIP qPCR primers | Targets | Forward | Reverse |
|-------------------|---------|---------|---------|
|                   | Up5k    | CCAAAGGCGTCACAACTCTT | CTCGCTGACTTCCTTCCTTC |
|                   | Endogenous hTERT promoter | GCGGCGGAGATTCTTCA | AGCACCTGCGCATG |
|                   | Transgenic hTERT promoter | GCGGCGGAGATTCTTCA | GTGCTTCACGCAACATTTCT |
|                   | RLucO   | ACCCTGGGTCTTTTCCAAC | CATTTCATCTGGACGTCCT |

| Lentiviral shRNA clones | shRNAs (Sigma-Aldrich) | TRC IDs | Oligo Sequences |
|-------------------------|------------------------|---------|-----------------|
|                         | Sp1a                   | TRCN0000020448 | CCAGCGCTGAGTGAGTGAATACATCTCGAAGATATTTCCCTACCCCGACAGCTTTTT |
|                         | Sp1b                   | TRCN0000020444 | CCAGCGAAGGTATTTCTCTACTGAGTAAGAGAAAATACCTTGGGTTTTTT |
|                         | Sp3a                   | TRCN0000020490 | CCAGCGAAGTATACTTCTATCTCGAAGTAAATCCCTACCCCTTCCTTTTT |
|                         | Sp3b                   | TRCN00000280370 | CCAGCGTGGTACTTACCTGGAATTACCTCGAAGTATTTCTACCCCTCTTCTCTTTTT |

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A.

- CRR9
- 10 kb
- GC-box
- E-box
- E2F
- hTERT

B.

- hTERT mRNA levels
- 3C167b 3C166a GM639 GM847 NHFs

C.

- Sp1 Actin
- Sp3 Actin

Figure 1
Figure 2

A.

Firefly Luciferase Activity

|          | Tel⁺ (3C167b) | Tel⁻ (GM847) |
|----------|---------------|--------------|
| pBT-255  | 2.0 ± 0.2     | 1.5 ± 0.1    |
| pBT-255gc| 1.8 ± 0.1     | 1.2 ± 0.05   |
| pGL3-control | 1.1 ± 0.05 | 0.8 ± 0.05   |
| pGL3-basic | 1.0 ± 0.05    | 0.7 ± 0.05   |

B.

|        | Tel⁺ | Tel⁻ |
|--------|------|------|
| pLXSN  | Sp1  | Sp1  |
| Sp1    |      |      |
| Actin  |      |      |
Figure 2

C.

[Graph showing Firefly Luciferase Activity for pBT-255, pBT-255gc, pGL3-control, and pGL3-basic with pLXSN and Sp1 conditions.]

D.

[Graph showing Firefly Luciferase Activity for shSp1a, shSp1b, and SCR conditions.]

17
Figure 3

A.

Rluc/Fluc

3C167b 3C166a GM639 GM847

B.

pLXSN

pLXSN-Sp1

Rluc/Fluc

Tel^+ Tel^−
Figure 3

C.

D.

Rluc/Fluc

H(wt)  H(gc)  H(gc5)

Tel+  Tel−

Rluc/Fluc

pLXSN  Sp1

H(wt)  H(gc)  H(gc5)  H(EboxD)
Figure 4

A. Chromosomal Acceptor Locus

BAC Reporter $H(\text{wt})$, $H(\text{gc})$, or $H(\text{gc5})$

Chromosomal Acceptor Locus

B. $\text{Rluc}/\text{Fluc}$

- TSA
  + TSA

| Tel$^+$ | Tel$^-$ |
|--------|--------|
| 10     | 0.01   |
| 10     | 0.01   |
Figure 4

D.

![Graph showing Rluc/Fluc for Mock, pLXSN, and Sp1 in Tel+ and Tel- conditions.](image)
Figure 5

A.

Relative hTERT promoter activity (Rluc/Fluc)

- TSA

+ TSA

H(wt)  H(gc)  H(gc5)  H(EboxD)

Tel⁺  Tel⁻
Figure 5

B.

- Up5k
- Endogenous Promoter
- Transgenic Promoter
- % Input

DNA binding proteins (Sp1, Sp3, Max, USF1, USF2, PolII) across different conditions (H(wt), H(gc), H(gc5)) for the specified promoters.
Figure 5

C. 

- **Up5k** 
  - **Endogenous Promoter** 
  - **Transgenic Promoter** 
  - **RlucO**
Figure 6

| Ab     | shSp1a | shSp1b | shSCR | Vector | Mock |
|--------|--------|--------|-------|--------|------|
| Sp1    |        |        |       |        |      |
| Actin  |        |        |       |        |      |
| Sp3    |        |        |       |        | None |
| Actin  |        |        |       |        |      |
Figure 6

B. hTERT mRNA levels

C. Rluc/Fluc

shRNAs

Sp1a, Sp1b, Sp3a, Sp3b

Day 3

Day 4

Mock, Vec, SCR

shRNAs
Figure 7

A.

- **Up5k**
- **Endogenous Promoter**
- **Transgenic Promoter**
- **RlucO**
Figure 8

- No hTERT transcription
- A permissive chromatin
- Active hTERT transcription

Tel+ cells

Chromatin remodeling
TF binding & nucleosomal remodeling

Sp1 family TFs
Other TFs
Figure 9

A.

Relative mRNA levels

|        | Tel\(^+\) | Tel\(^-\) |
|--------|-----------|-----------|
| p53    | 5         | 3         |
| c-Myc  | 4         | 2         |
| Max    | 5         | 3         |
| Sp1    | 3         | 2         |

![Graph showing relative mRNA levels for Tel\(^+\) and Tel\(^-\) conditions.](image)

Control  TSA

B.

|          | Tel\(^-\) | Tel\(^+\) |
|----------|-----------|-----------|
| TSA      | -         | +         |
| c-Myc    | -         | +         |
| Actin    | -         | +         |

![Western blot showing c-Myc and Actin expression in Tel\(^-\) and Tel\(^+\) conditions.](image)
Human telomerase reverse transcriptase (hTERT) transcription requires Sp1/Sp3 binding to the promoter and a permissive chromatin environment

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