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Regulation of the human catalytic subunit of telomerase (hTERT)

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Abstract

Over the past decade, there has been much interest in the regulation of telomerase, the enzyme responsible for maintaining the integrity of chromosomal ends, and its crucial role in cellular immortalization, tumorigenesis, and the progression of cancer. Telomerase activity is characterized by the expression of the telomerase reverse transcriptase (*TERT*) gene, suggesting that *TERT* serves as the major limiting agent for telomerase activity. Recent discoveries have led to characterization of various interactants that aid in the regulation of human *TERT* (*hTERT*), including numerous transcription factors; further supporting the pivotal role that transcription plays in both the expression and repression of telomerase. Several studies have suggested that epigenetic modulation of the *hTERT* core promoter region may provide an additional level of regulation. Although these studies have provided essential information on the regulation of *hTERT*, there has been ambiguity of the role of methylation within the core promoter region and the subsequent binding of various activating and repressive agents. As a result, we found it necessary to consolidate and summarize these recent developments and elucidate these discrepancies. In this review, we focus on the co-regulation of *hTERT* via transcriptional regulation, the presence or absence of various activators and repressors, as well as the epigenetic pathways of DNA methylation and histone modifications.

Keywords

Human telomerase reverse transcriptase (hTERT); Telomerase; Cancer; Gene regulation; Telomeres

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1. Introduction

Telomerase is a cellular ribonucleoprotein with reverse transcriptase activity (Liu, et al., 2004) that facilitates the capping of the ends of eukaryotic chromosomes via telomeres (Cukusić et al., 2008). The main function of these telomeres is to maintain chromosomal integrity and genome stability (Blackburn, 2001) via tandem hexameric repeats of a 5′-TTAGGG-3′ sequence ending in a 3′ single-stranded overhang or the G-strand overhang (Moyzis et al., 1988; Wellinger et al., 1997; Makarov et al., 1997; as reviewed by De Boeck et al., 2009). Telomerase remains inactive in most somatic cells but can be readily detectable in germ cells and other self-renewing tissues (Liu et al., 2004). In normal somatic cells, the erosion of telomeres is caused by incomplete replication at telomeric ends, leading to a loss of approximately 50 to 200 bp of telomeric DNA at each cell division until replicative senescence is reached (Chiu et al., 1997; Harley et al., 1990; Qi et al., 2011) (Fig. 1). However, in the presence of telomerase, telomere length is maintained via the addition of hexameric repeats that cap the ends of linear chromosomes and allows their evasion of replicative senescence (Liu et al., 2004). The dysregulation of telomerase leads to its activation in approximately 90% of human cancers, irrespective of tumor type (as reviewed by Kyo et al., 2008). This suggests that normal human somatic cells utilize regulatory factors to suppress the activity of telomerase; however, upon cellular immortalization, this regulation is either deviant or missing (Collins et al., 2002; Qi et al., 2011).

Recent studies have identified several components of telomerase: the telomerase RNA (TER) molecules whose secondary structure is well defined; telomerase reverse transcriptase (TERT), the catalytic subunit; Es1p and Es3p which are categorized as additional protein subunits, more specifically the two units of the Ku heterodimer; as well as a vast majority of assembly and maturation proteins that contribute to the telomerase enzymatic complex (Liu et al., 2004). Dyskerin (DKC1) (Cohen et al., 2007) and telomerase protein component 1 (TEP1) (Saito et al., 1997) have also been identified as components of telomerase. However, it has been shown that only hTER and hTERT are necessary for the reestablishment of telomerase activity (Ishikawa, 1997; Weinrich et al., 1997; Beattie et al., 1998; Liu et al., 2004). On the other hand, recent evidence suggests that only hTERT is needed to restore telomerase activity in telomerase-negative normal cells, such as epithelial cells and human fibroblasts (Artandi et al., 2002; Gonzalez-Suarez et al., 2002; Gonzalez-Suarez et al., 2001; Stewart et al., 2002; Qi et al., 2011), and the ectopic expression of hTERT along with activated oncogenes results in tumorigenesis (Hahn et al., 1999; Qi et al., 2011). Although hTERT is tightly regulated during cellular differentiation and is expressed at very low levels in normal somatic cells (Masutomi et al., 2003), hTER is widely expressed in the majority of cell types including those that are telomerase-negative (Meyerson et al., 1997; Nakamura et al., 1997; Liu et al. 2004). This suggests the presence of additional mechanisms that aid in the protection of chromosomal ends. A study done by Buckhovich and Greider (1996) showed that lymphocytes, in particular, exhibit telomerase activity in response to stimulation (Chebel et al., 2009). Regulation of *hTERT* and subsequent telomerase expression in these specific cells is thought to occur via the inhibition of rapamycin during the G1 phase of the cell cycle (Chebel et al., 2009).

Studies have shown that telomeric ends form higher-order structures called T-loops that potentially protect these termini from the DNA repair system as well as regulate the access of telomerase to the telomere (Griffith et al., 1999; as reviewed by De Boeck et al., 2009). The TTAGGG duplex repeats found at the ends of mammalian telomeres are bound by two related proteins: TRF1 and TRF2, the TTAGGG repeat binding proteins (Chong et al., 1999; Bilaud et al., 1997; Broccoli et al., 1997; Griffith et al., 1999). TRF2 plays an essential role in both telomere end protection (van Steensel et al., 1998; Karlseder et al., 1999; Karlseder et al., 2002; as reviewed by De Boeck et al., 2009) and T-loop formation (Griffith et al.,

1999). Subsequent studies have shown that the TRF2 complex promotes T-loop formation via its interaction with telomere overhang, notably the 3′ guanine-rich termini (Griffith et al., 1999; Stansel et al., 2001; Khan et al., 2007; as reviewed by De Boeck et al., 2009). Thus, this system could potentially play an essential role in the protection of telomeres via the formation of higher-order structures, concealing overhang and preventing the degradation of telomeres at DNA damage checkpoints (De Boeck et al., 2009). Further elucidation of these mechanisms is crucial in understanding the regulation of hTERT both *in vivo* and *in vitro* and the development of proactive anticancer therapies.

2. The *hTERT* promoter region

Transcription, alternate mRNA splicing, phosphorylation, and the maturation and modification of *hTERT* and *hTER* have all been shown to play vital roles in the regulation of telomerase activity (Cong et al., 2002; Cukusić et al., 2008). However, it is thought that the *hTERT* promoter is the most important regulatory element of telomerase expression (Cukusić et al., 2008). Cong et al. (1999) and Bryce et al. (2000) showed that the *hTERT* gene is located on the short arm of human chromosome 5 (5p15.33), more than 2 Mb away from the telomere (Leem et al., 2002). A recent study has also discovered an approximate 7 Mb telomerase repressor region located on the short arm of chromosome 3 (3p21.3) (Abe et al., 2010). The *hTERT* gene consists of 15 introns and 16 exons and is over 40 kb in length (Cukusić et al., 2008). Although rich in CpG dinucleotides and Sp1 sites, the hTERT promoter lacks both TATA and CAAT boxes (Horikawa et al., 1999; Takakura et al., 1999). The *hTERT* core promoter spans 330 bp upstream of the translational start site and 37 bp of exon 2 (Cong et al., 1999; as reviewed by Cukusić et al., 2008). Moreover, this regulatory region as well as upstream sequences interacts with both positive and negative regulators of hTERT via an abundance of transcriptional binding sites (Cukusić et al., 2008) (Table 1), further suggesting the compelling role of transcription in the regulation of hTERT.

Recent discovery has shown that the G-rich sequences located within the *hTERT* promoter have a strong potential to form G-quadruplexes (Simonsson, 2001; Davis, 2004; Burge, 2006; Patel, 2007), which are helical structures composed of four-strands and formed via the stacking of GGGG tetrads (Lim et al., 2010; Gellert, 1962). Lim et al. (2010) discovered the coexistence of two major intramolecular G-quadruplex conformations within the G-rich region of the hTERT promoter, *GTERT-060*: Form 1, an intramolecular (3 + 1) G-quadruplex and Form 2, a propeller-type parallel-stranded G-quadruplex. Quadruplex formation within the telomeric G-rich overhang has also been shown to prevent its elongation by telomerase (Zahler et al., 1991), negatively impacting *c-Myc* expression and in turn the expression of *hTERT* (Grand et al., 2002), and altering the splicing pattern of *hTERT* RNA (Gomez et al., 2004) (Lim et al., 2010). In addition, a study done by Gros et al. (2008) revealed a quadruplex ligand that binds to *hTER* at its quadruplex-forming motif and can alter telomerase activity (Lim et al., 2010). These studies present a novel mechanism by which telomerase activity can be down-regulated or even inhibited in cancer models via G-quadruplex ligands that target the guanine-rich region at telomeric ends (Gros et al., 2008).

3. The *hTERT* promoter and associated transcription factors

After the successful cloning of the *hTERT* 5′-promoter region (Takakura et al., 1999; Horikawa et al., 1999; Cong et al., 1999; as reviewed by Kyo et al., 2008), studies were able to show an up-regulation of transcriptional activity in cancer cells and a lack of detectable activity in most normal cells via transient expression assays of the *hTERT* gene (Takakura et al., 1999; as reviewed by Kyo et al., 2008). This 5′ regulatory region contains numerous binding sites for a variety of transcription factors, including both activators and repressors of *hTERT* (Renaud et al., 2006) (Table 1). Renaud et al. (2006) found that there are 5′ exonic

regions that may inhibit the transcriptional activity of *hTERT* and serve to regulate its expression. This finding is promising because the CCCTC-binding factor (CTCF) binds to these regions in telomerase-negative cells but not in telomerase-positive cells (Renaud et al., 2005), suggesting that CTCF may bind downstream of the transcriptional start site (Klenova et al., 1993; Lutz et al., 2000) and act as a repressor of hTERT in normal cells (Renaud et al., 2006).

A recent study showed that the proximal E-box, located +22 to +27 upstream of the transcriptional start site, is a repressive cis-element of the *hTERT* promoter in certain cell lines (Horikawa et al., 2002). The repressive nature of the E-box is thought to be associated with an interaction with the Mad1-Max complex in normal human somatic cells (Oh et al., 2000). Furthermore, several investigators have shown that c-Myc acts as a key regulator of *hTERT* transcription during carcinogenesis via its binding to the E-box and subsequent activation of transcription (Cong et al., 1999; Greenbery et al., 1999; Wang et al., 1998; Wu et al., 1999; as reviewed by Kyo et al., 2008). However, it should be noted that other studies have found that c-Myc does not necessarily affect *hTERT* expression in some cancer cells (Kirkpatrick et al., 2003; Günes et al., 2000). GC-boxes (GGGCGG) are another characteristic sequence of the *hTERT* promoter (as reviewed by Kyo et al., 2008). Xu et al. (2001) proved by electrophoretic mobility shift assay (EMSA) that Sp1, an activator of *hTERT* (Kyo et al., 2000), binds to these CG-boxes. Likewise, Filippova et al. (1996) showed that CTCF acts to inhibit *hTERT* expression when bound downstream of the transcriptional start site and preferentially to the CG boxes (Renaud et al., 2006). In this review, we will attempt to elucidate and summarize these findings and present the roles that transcription plays in the regulation of *hTERT*.

3.1 Activators of hTERT

3.1.1 Transcription factors—There a several transcription factors that act a regulators of *hTERT* (Table 1). Sp1 is a zinc finger transcription factor (Suske, 1999) that has been shown to bind to the five GC-boxes on the TATA-less *hTERT* promoter (Cukusić et al., 2008) and activate *hTERT* (Kyo et al., 2000). Kyo et al. (2000) also localized these Sp1 binding sites between two E-boxes 110 bp upstream of the transcriptional start site. Luciferase reporter assays performed by Cong and Bacchetti (2000) showed that any mutations in the Sp1 binding sites drastically reduced *hTERT* promoter activity, further suggesting the regulatory role of Sp1 in the transcription of *hTERT*. However, it should be noted that because Sp1 is often universally expressed in normal cells, it is evident that Sp1 alone does not lead to the activation of *hTERT* (Horikawa et al., 2002). Sp1 has also been suggested to cooperate with the oncogene, *c-Myc*, to activate the transcription of the *hTERT* gene (Kyo et al., 2000). Liu et al. (2009) suggested a common mechanism in proliferative cancer cells by which Sp1 stimulates telomerase expression via the transcriptional function of the MBD1-containing chromatin-associated factor 1 (MCAF1). In the study, MCAF1 was shown to have a direct relationship with Sp1, and the depletion of either Sp1 or MCAF1 down-regulated the *TERT* and *TER* genes in cultured cells, resulting in decreased activity of telomerase (Liu et al., 2009). The high-mobility group A2 (*HMGA2*) gene, most frequently amplified in human cancers, has also been shown to regulate the expression of *hTERT* by interacting with Sp1 and impeding the recruitment of histone deacetylase 2 (HDAC2) to the *hTERT* proximal promoter (Li et al., 2011). In turn, this impediment stimulates hTERT expression and telomerase activity via the enhanced acetylation of histone H3-K9 (Li et al., 2011). Moreover, the study showed that *HMGA2* partially replaced *hTERT* during the tumorigenic transformation of normal human fibroblasts (Li et al., 2011). A study done by Chebel et al. (2009) suggested a synergistic relationship between the nuclear factor of activated T cells (NFAT) and Sp1 in the transcriptional regulation of *hTERT*. The research showed that simultaneous mutations of both the −40 NFAT-responsive element and one or more Sp1-

binding sites led to a greater decrease in *hTERT* promoter activity when compared to single mutations, suggesting NFAT as activator of *hTERT* apart and in the presence of Sp1, mainly through a consensus binding site localized within the core promoter (Chebel et al., 2009). Moreover, Chebel et al. (2010) proposed the activation of *hTERT* by NFAT at two levels: directly, at the level of transcription (Chebel et al., 2009) and indirectly via the activation of c-Myc (Buchholz et al., 2006) (Fig. 2). Sequential induction of Nuclear Factor-kappa B (NF-κB) and c-Myc has been suggested to aid human epidermal growth factor receptor 2 (HER2) in the activation of *hTERT* following irradiation (Papanikolaou et al., 2011). It was determined that the knockdown of HER2 led to the down-regulation of NF-κB and c-Myc-mediated *hTERT* and telomerase activity in human breast cancer cells (Papanikolaou et al., 2011). Deng et al. (2007) identified the activating enhancer binding protein-2β (AP-2β) as a unique transcriptional activator of *hTERT* in human lung cancer cells. Furthermore, it was shown that the inhibition of AP-2β down regulated telomerase activity, accelerated telomere degradation, and inhibited tumorigenesis, suggesting this pathway as a potentially novel therapeutic target in certain cancer lines (Deng et al., 2007).

Signaling and regulatory pathways with no component factors binding to the 5′-regulatory region have potentially decisive roles in the transcriptional regulation of *hTERT* through their regulation of critical *hTERT* transcription factors such as c-Myc and Mad1. In particular, the TGF-β pathway has been shown to down-regulate *hTERT* expression by increasing Mad1 and suppressing c-Myc (Wu et al., 2009; Chen et al., 2002; Hein et al., 2011). Several investigators demonstrated that the P13K/Akt and MAP kinase pathways up-regulate *hTERT* transcription via the phosphorylation of Mad1, leading to its ubiquitin-mediated proteolysis (Zhu et al., 2008; Chou et al., 2009). The role of the P13K/Akt pathway may, in fact, be central to the process of *hTERT* dysregulation and cellular immortalization. The pathway has been shown to potentially inhibit the TGF-β pathway by Jab1 activation and SMAD4 degradation, activate the NF-κB pathway, disrupt cell cycle control and advance cell cycle progression by either the degradation or down-regulation of p53, p21, and p27 (Ogawara et al., 2002; Bai et al., 2009; Li and Sarkar, 2002; Tomoda et al., 2002; Oh et al., 2006; Hsu et al., 2007; Johnson et al., 2010; Wan et al., 2002). A growing body of evidence now supports the existence of a positive feedback loop whereby the expression of *hTERT* activates the P13K/Akt pathway, which in turn initiates multiple mechanisms that increase *hTERT* expression and/or inactivate *hTERT* expression restraints (Lai et al., 2007; Perrault et al., 2005; Takano et al., 2008; Smith et al., 2003; Li et al., 2011; Farwell et al., 2000; Sasaki et al., 2009)(Table 1).

3.1.2 Oncogenes—Although *c-Myc* is an oncogene that is generally involved in the management of cellular proliferation, differentiation, and apoptosis in normal human cells, it is also often associated with the development of human cancers when mutated or over expressed and in instances of chromosome translocation and gene amplification (Cukusić et al., 2008). c-Myc protein levels are known to be elevated in undifferentiated and most neoplastic and transformed cells (Lie et al., 2001) but are minimal in differentiated somatic cells (Günes et al., 2000). Xu et al. (2001) demonstrated that c-Myc binds to the E-boxes (5′-CACGTG-3′) at the *hTERT* proximal promoter in exponentially proliferating HL60 cells, suggesting that the E-box occupancy by c-Myc is responsible for the activation of *hTERT* in this particular cell line. It has been shown that the product of *c-Myc* often complexes with Max protein to form a heterodimer (c-Myc/Max) that activates gene transcription (Blackwood and Eisenman, 1991; Liu et al., 2004). Kyo et al. (2000) demonstrated that the Myc/Max heterodimer binds to the E-box in the *hTERT* promoter and is essential in transactivation. In addition, Kyo et al. (2000) suggested a coactive function of c-Myc and Sp1 as important regulators of *hTERT* expression via the positive correlation between the transcriptional activity of *hTERT* and the expression of c-Myc and Sp1. However, because a large majority of these studies used overexpressed c-Myc when measuring *hTERT* promoter

activity, it holds unclear as to the regulatory role that c-Myc plays in the transcription of *hTERT* when bound endogenously at its promoter (as reviewed by Kyo et al., 2008). Recent findings have challenged the exclusive role of Myc/Max in E-box dependent binding and activation of *hTERT* via the recognition of other E-box-binding proteins that regulate *hTERT* transcription, notably upstream stimulatory factor (USF) 1 and 2 (Goueli et al., 2003). The study suggested that the binding of USF1/2 precludes the binding of Myc/Max to the E-box in the *hTERT* promoter and indicated that USF1/2 binds more avidly to the *hTERT* E-boxes than does Myc/Max (Goueli et al., 2003). This regulation of *hTERT* at the E-boxes will be further discussed in combination with the repression of *hTERT* via the binding of Mad1.

3.1.3 Hormones—Several studies have shown the up regulation of telomerase activity in breast and prostate cancer cells by estrogen (Kyo et al., 1999; Misiti et al., 2000; Nanni et al., 2002; Gao et al., 2003). The *hTERT* promoter contains two estrogen responsive elements; one at −2754 bp and the other at −950 bp upstream of the ATG start site (Cukusić et al., 2008). Some groups have found that estrogen receptor-α (ERα) binds to the estrogen-response element (ERE) in the *hTERT* promoter region and activates *hTERT* transcription, thus supporting the idea that estrogen up regulates telomrase activity (Kyo et al., 1999; Misiti et al., 2000; as reviewed by Kyo et al., 2008). Others have shown that ERα, when bound to the upstream *hTERT* regulatory region and activated by bound estrogen, can potentially block the repressive TGF-β pathway via interactions with the TGF-β receptor or with AP1 (Cherlet and Murphy, 2007; Welboren et al., 2009; Tee et al., 2004) (Fig. 2). Moreover, Kimura et al. (2004) demonstrated the activation of *hTERT* via phosphorylation, mediated by Akt signaling, in which *hTERT* is phosphorylated in an Akt-dependent manner. Tamoxifen, a selective modulator of ER, has also been shown to regulate *hTERT* expression in a cell-specific manner (Wang et al., 2002) by inhibiting the growth of breast cancer cells and down regulating the expression of *hTERT* mRNA in the presence of estrogen (as reviewed by Kyo et al., 2008). However, tamoxifen was shown to activate *hTERT* and stimulate the growth of endometrial cancer cells in both the absence and presence of estrogen (E2) (as reviewed by Kyo et al., 2008), solidifying its cell-specific manner. Previous studies conflicted on whether the induction of *hTERT* by estrogen was c-Myc dependent (Misiti et al., 2000; Kyo et al., 1999), but later studies by Kirkpatrick et al. (2003) and Wisman et al. (2003) agreed on the regulatory effects of estrogen on telomerase activity in certain tissues, markedly those targeted by estrogen. These studies did not, however, conclude whether the activation of *hTERT* is direct or a result of the up regulation of cMyc by estrogen (Cukusić et al., 2008). Interestingly, research of obese women has shown that adiposity increases the circulating levels of estrogen in adipose tissue via the conversion of androgens to estrogens by aromatase (Potischman et al., 1996), resulting in the stimulation of mammary epithelial cell mitosis and tumorigenesis (Han et al., 2005). Recent studies have drawn a link between leptin, an adipose derived hormone, obesity, and breast cancer risk (Rose et al., 2007; Rahmati-Yamchi et al., 2011). A correlation was observed between high serum levels of leptin and *hTERT* mRNA levels (Rahmati-Yamchi et al., 2011). *In vitro* studies have shown that in MCF-7 breast cancer and HepG2 liver carcinoma cell lines, *hTERT* is up regulated by leptin (Ren et al., 2010; Stefanou et al., 2010). Androgen has also been shown to be an activator of *hTERT* in androgen-sensitive prostate cancer cells (Guo et al., 2003; as reviewed by Kyo et al., 2008).

3.2 Repressors of hTERT

3.2.1 Transcription factors—CCCTC-binding factor (CTCF) is a ubiquitously expressed 11-zinc finger protein that binds to the *hTERT* proximal exonic region (Renaud et al., 2005) and is thought to be the only factor that can negatively regulate the transcription of *hTERT* irrespective of cell type (Renaud et al., 2005). Studies have shown that CTCF plays a pivotal role in the transcriptional activation of the *APPB* promoter (Vostrov et al., 1997), the

imprinting control of the H19 region (Kanduri et al., 2000), and the silencing of cMyc (Filippova et al., 1996) However, a more recent study demonstrated that CTCF-binding at the cMyc locus does not repress the transcriptional activity of cMyc, but instead is required for its protection from DNA methylation (Gombert and Krumm, 2009) (Fig. 2). In one model, CTCF was revealed to be bound to *hTERT* in cells that did not express *hTERT* but not in telomerase-positive ones (Renaud et al., 2005), strongly suggesting that the binding of CTCF may serve as an important repressive mechanism of *hTERT* in normal cells. CTCF is known to bind directly to SIN3A, which recruits HDACs and thus prevents transcription (Lutz et al., 2000). However, Renaud et al., (2010) revealed evidence that BORIS (Brother of the Regulator of Imprinted Sites) may exert an opposing effect by opening chromatin around the transcriptional start site of *hTERT*, allowing for the expression of *hTERT*. In *hTERT*-negative BJ fibroblasts and HLF/hTERT cells, CTCF was demonstrated to bind to the first exon of the *hTERT* gene; however, CTCF did not exhibit binding in HeLa and SW480 tumor cell lines (Renaud et al., 2006). This study showed methylation patterns in the CpG islands and core promoter of *hTERT* to be the mechanism by which CTCF is prevented from binding in telomerase-positive cells (Renaud et al., 2006), implying an epigenetic role in the expression or repression of *hTERT*. Lee et al. (2003) demonstrated that IRF1 is a mediator for interferon-γ-induced inhibition of both hTERT expression and telomerase activity in cervical cancer cells. IRF1 increased by two-fold in the presence of over expressed WT1D, indicating its possible inhibitory effect on *hTERT* promoter activity (Sitaram et al., 2010). Jun and Fos family proteins form a heterodimeric complex to create transcriptional activator protein 1 (AP-1) which has been shown to lead to the transcriptional suppression of *hTERT* in cancer cells (Takakura et al., 2005). Chromatin immunoprecipitation (ChIP) analysis revealed the binding of JunD and c-Jun to two putative AP-1 binding sites between −2000 and −378 of the *hTERT* promoter region, suggesting that AP-1 directly associates with the *hTERT* promoter (Takakura et al., 2005). Ap1 has also been reported to enhance the repressive activity of the TGF-β pathway (Cherlet and Murphy, 2007). Interferon-β (IFN-β) signaling was shown by Lee et al. (2010) to repress telomerase activity and *hTERT* transcription in ovarian cancer. In addition to down regulating *hTERT* mRNA expression, IFN-β also induces p21 expression, independently of p53 (Lee et al., 2010). Shats et al. (2004) reported that p21 and E2F mediate the repression of *hTERT* via p53. Makorin-1 (MKRN1) is an E3 ubiquitin ligase that targets hTERT for proteasome processing (Kim et al., 2005). A study done by Salvatico et al. (2010) proposed an inverse relationship between the MKRN1 protein and telomerase activity, following terminal differentiation in HL-60 cells. This study is consistent with the idea that during differentiation or cell cycle arrest when telomerase action at chromosomal ends is no longer needed, MKRN1 represents an elimination pathway to rapidly reduce telomerase activity (Salvatico et al., 2010). Recently, *PITX1* was identified as a repressor of *hTERT* promoter activity that binds directly to the promoter region (Qi et al., 2011). Several studies have reported reduced *PITX1* expression in various types of human cancer, including gastric, bladder, and colon cancers, (Chen et al., 2007; Chen et al., 2008), strongly supporting the idea that *PITX1* also plays a crucial role in the neoplastic development (Qui et al., 2011).

Research has shown that Mad1 can act as a transcription factor that binds with the E-box of the *hTERT* promoter and regulates its activity (Casillas et al., 2003). Mad1 also has the potential to interact and form a dimer with c-Myc at the specific E-box site sequence 5′-CACGTG-3′ and suppress the transcription of target genes (James et al., 2002). Furthermore, Mad1 has been shown to suppress *hTERT* transcription via competition with c-Myc for the CACGTG binding sites of the *hTERT* promoter (Zou et al., 2005). The research done by Gunes et al. (2000) demonstrated that the Myc-Mad1 dimer binds more easily to the E-box sites than does c-Myc or Mad1 alone. This additive relationship was shown to suppress *hTERT* expression in bladder cancer cells and illustrates a partial mechanism by which *hTERT* is regulated during carcinogenesis (Zou et al., 2005). A study by Won et al.

(2002) found that Sp1 and Sp3 interact with histone deacetylase (HDAC) in human fibroblasts and play an important role in the repression of the *hTERT* promoter endogenously. This suggests that both Sp1 and Sp3 interact directly with the *hTERT* promoter by recruiting HDAC and repressing the transcription of *hTERT* in normal somatic cells (Won et al., 2002).

3.2.2 Tumor suppressors—p53 is likely the most characterized of the tumor suppressors. The gene acts as a tumor suppressor via its ability to induce cell cycle arrest and apoptosis in response to various stress stimuli (Asker et al., 1999; Cukusić et al., 2008). This suppressor gene is mutated in 50% of human tumors (Ko and Prives, 1996). Mechanisms of cell cycle inhibition and cancer prevention by p53 include the transcriptional activation of p21, inhibition of basal transcription factor TFIID, and the suppression of cyclin/cdk complex activity (Shats el al., 2004; Lai et al., 2007; Henderson et al., 2000) (Fig. 2).. Kanaya et al. (2000) showed that the *hTERT* gene has two p53 binding motifs upstream of the 5′ core promoter region, and an over expression of p53 and its subsequent binding with the help of transcription factor Sp1 at these two motifs leads to the repression of the *hTERT* promoter (Lai et al., 2007). Lai et al. (2007) also demonstrated that a knockdown of *hTERT* in HEK 293 cells resulted in elevated p53 transcription as well as a decrease in cellular proliferation, further supporting the antagonistic role of p53 in *hTERT* expression (Fig. 2). Shats et al. (2004) indicated that the DNA-binding domain of p53 is required for the repression of *hTERT*, but no direct binding of p53 to the *hTERT* core promoter was observed. In the absence of p53, an atypical E2F-binding site in the 5′ untranslated region of the *hTERT* promoter upregulated *hTERT* expression; however, *hTERT* expression was down regulated in the presence of p53 (Cukusić et al., 2008; Shats et al., 2004). Results from Shats et al. (2004) showed a repression of telomerase activity and *hTERT* expression in OVCAR and SKOV3 cell lines by p21, suggesting IFN-β/p21 signaling could possibly down regulate both *hTERT* expression and telomerase activity via a p53-independent pathway.

Inactivation of the retinoblastoma protein Rb is common in many types of cancers (Murphree and Benedict, 1984). pRb binds to and inhibits transcription factors of the E2F family (Cukusić et al., 2008). Rb creates a complex and acts as a growth suppressor when bound to E2F, causing cell cycle arrest and recruiting HDAC to further suppress DNA synthesis (Cukusić et al., 2008). Zheng and Lee (2002) showed that Rb is active when in a hypophosphorylated state and inactive when phosphorylated, supporting several studies that indicated a down-regulation of telomerase activity in human carcinoma cell lines via an over expression of hypophosphorylated Rb (Xu et al., 1997; Nguyen and Crowe, 1999). Meeran et al. (2011) revealed that the acetylated green tea polyphenol, pEGCG, inhibits *hTERT* expression partially through the binding of E2F-1 and Mad1 to the *hTERT* promoter in both ER (+) and ER (−) breast cancer cells.

Wilms' tumor 1 (WT1) is a tumor suppressor gene, potentially oncogenic, that acts as an important regulator of cellular processes, particularly cell growth and development, as well as a strong transcriptional regulator of *hTERT* (Sitaram et al., 2010). WT1 has been shown to repress the transcription of *hTERT* in specific cell types, notably the virally transformed human embryonic kidney 293 cells identified by Oh et al. (1999). There is strong indication that there are multiple pathways for *hTERT* regulation by WT1 in clear cell renal cell carcinoma (ccRCC), though the repression of the *hTERT* promoter generated by WT1 is complex (Sitaram et al., 2010). WT1 has been shown to bind to the promoters of *hTERT*, *c-Myc*, and *SMAD3*, and suppress c-Myc at both a transcriptional and protein level; suppress *hTERT* expression; and up-regulate SMAD3 and Jun (Sitaram et al., 2010). Furthermore, direct repression of *hTERT* by SMAD3 via TGF-β has also been reported (Lacerte et al., 2008).

4. Epigenetic mechanisms regulating hTERT

The cluster of CpG sites found within the *hTERT* promoter has prompted many researchers to believe that methylation plays a key role in the regulation of hTERT (Kyo et al., 2008). However, contradictory results have been reported. Some groups showed that methylation of the *hTERT* promoter resulted in the silencing of the gene (Shin et al., 2003; Lopatina et al., 2003; Liu e al., 2004), while others have drawn no significant correlation between the methylation of the *hTERT* promoter and subsequent expression of the gene (Devereux et al., 1999; Dessain et al., 2000). Meeran et al. (2008) and Renaud et al. (2007) have shown that in most cancer cells, the regulatory region of *hTERT* is hypermethylated, which is associated with increased expression, whereas demethylation of this region inhibits *hTERT* transcription. This pattern is contrary to what commonly occurs during the regulation of genes via methylation, in which methylation of the cytosines in a promoter region generally inhibits gene transcription (Kikuno et al., 2008; Majid et al., 2008; Meeran et al., 2010). Hypomethylation of the *hTERT* promoter can be seen in certain *hTERT* negative cells, markedly those that have not been differentiated or transformed, suggesting a mechanism independent of promoter methylation by which these cells tightly repress the transcription of *hTERT* (Dessain et al., 2000; Liu et al., 2004; Lopatina et al., 2003; Shin et al., 2003). Zinn et al. (2007) indentified little to no methylation of alleles around their transcriptional start sites in telomerase-positive cancer cells, despite an abundance of methylation in more upstream regions. This suggests that the absence of methylation at and around the transcriptional start site allows for the transcription and expression of *hTERT*, which shows some consistency with the usual dynamics of gene expression (as reviewed by Kyo et al., 2008).

Research has shown that total methylation of *hTERT* inhibits transcription and that a region of the *hTERT* minimal promoter must be hypomethylated in order for transcription to proceed (Renaud et al., 2007). CTCF, a repressor of *hTERT* activity, has shown to be regulated through the methylation of its recognition sequence (Renaud et al., 2007). In this study, ChIP assays revealed that CTCF binds to the first exon of *hTERT* when its CpG island is not methylated and no longer binds when its regulation sequence is methylated (Renaud et al., 2007). Therefore, it can be postulated that hypermethylation of the CTCF recognition sequence suppresses the factor's repressor activity, and that the main purpose of methylating the *hTERT* CpG island is likely to prevent the binding of CTCF and allow for the transcription of *hTERT* (Renaud et al., 2007). Choi et al. (2010) found that trichostatin A (TSA) induced the demethylation of site-specific CpGs on the *hTERT* promoter via the down regulation of DNA methyltransferase 1 (DNMT1). Within this demethylated region is a binding site for CTCF, located between the 31st and 33rd CpGs; ChIP analysis revealed that the demethylation of these particular CpG sites by TSA promoted CTCF binding to the *hTERT* promoter and led to the repression of *hTERT* transcription. Previous studies have shown that certain dietary compounds, notably genistein and EGCG, down-regulate DNMTs which is directly associated with the repression of *hTERT* via *hTERT* promoter demethylation in breast cancer cells (Berletch et al., 2008; Li et al., 2009; Meeran et al., 2011). It has also been suggested that EGCG-induced down-regulation of DNMTs is not only involved in the regulation of *hTERT* in the process of anti-carcinogenesis, but also in enhancing the binding of methylation-sensitive transcription factors such as E2F-1 to the *hTERT* promoter region (Crowe et al., 2001; Meeran et al., 2011). Furthermore, Meeran et al. (2010) found that sulforaphane (SFN), an isothiocyanate found in cruciferous vegestables, aids in the down-regulation of DNMT1 and DNMT3a, resulting in site-specific CpG demethylation in the *hTERT* promoter and thereby facilitating the binding of CTCF and subsequent repression of *hTERT*. A study by Iliopoulos et al. (2009) revealed that *hTERT* expression levels were inversely related with DNA methylation levels in hepatocellular carcinoma (HCC) and normal tissues. Moreover, *hTERT* expression was determined to be

regulated by DNA methylation and histone H3-K9 modifications, affecting the ability of cMyc to bind to E-box 1 on the *hTERT* promoter and further supporting the proposal that *hTERT* is regulated both epigenetically via mechanisms of DNA methylation and histone modifications and by cMyc in certain cancers (Iliopoulos et al., 2009).

Modification of histones by acetylation/deacetylation is known to regulate the structure of chromatin and thereby affect gene transcription (Stein et al., 2000; as reviewed by Kyo et al., 2008). Histone acetylases (HATs) and histone deacetylases (HDACs) generally cause the acetylation and deacetylation of chromatin, respectively, and thus play pivotal roles in the regulation of *hTERT* expression (Choi et al., 2009; Meeran et al., 2010). Meeran et al. (2010) revealed that SFN-induced hyperacetylation of the *hTERT* promoter facilitated the binding of MAD1, CTCF, and several other repressor proteins to the *hTERT* regulatory region. One study indicated that HDAC inhibitors activate the *hTERT* promoter in normal cells in a Sp1-dependent manner (as reviewed by Kyo et al., 2008). A role for histone methylation in the regulation of *hTERT* has also been demonstrated. A recent study reported that highly trimethylated H3-K4 is associated with active transcription of the *hTERT* gene in telomerase-proficient tumor cells (Atkinson et al., 2005). Furthermore, Liu et al. (2007) found that SET-and MYND-domain-containing protein-3 (SMYD3) are highly important in the regulation of the *hTERT* promoter. SMYD3-mediated trimethylation of histone H3-K4 may lead to the further recruitment of HAT via its function as a recruitment element for the consequent binding of transcription factors to the *hTERT* promoter (as reviewed by Kyo et al., 2008). A recent study by Mao et al. (2011) showed that Sirt1, a NAD-dependent class III histone deacetylase, interacts with the C-terminus of cMyc, causing its deacetylation both *in vitro* and *in vivo*. Furthermore, this deacetylation of cMyc by Sirt1 was shown to promote its association with Max, an essential partner for cMyc activation, and facilitate cMyc transactivation activity on the promoter of *hTERT*, suggesting a potential role of Sirt1 in tumorigenesis, notably in K562 human leukemia cells (Mao et al., 2011). Epigenetic control and modulation of the *hTERT* promoter via methylation and acetylation qualifies as a promising method in understanding the regulation of *hTERT*, though further research is needed to elucidate these mechanisms.

5. Conclusion

Recent studies have presented a broad range of mechanisms by which hTERT is controlled and regulated in both cancer and normal cell lines. Transcriptional regulation via the recruitment of activators or repressors is most commonly associated with the expression or silencing, respectively, of the *hTERT* gene. Epigenetics has also been shown to play a pivotal role in the regulation of *hTERT* through various methylation and acetylation mechanisms. However, the abundance of regulatory models reveals the need for further research in determining how hTERT expression and telomerase activity are regulated *in vivo* and *in vitro*.

Understanding the mechanisms of hTERT regulation is imperative for many reasons. Telomerase plays in important role in the maintenance, protection, and stabilization of linear chromosomes (Cukusić et al., 2008). Unfortunately, this diversity can lead to numerous opportunities for cancers to activate *hTERT* during tumorigenesis and escape cell senescence (Cukusić et al., 2008). Breakthroughs in the discovering the roles of various regulatory elements of hTERT have provided new approaches in the treatment of certain cancers and a better understanding of tumorigenesis. Various dietary compounds, such as genistein, EGCG, and sulforaphane show promising avenues by which *hTERT* is down-regulated and cellular apoptosis induced in human breast cancer cells (Meeran et al., 2010; Meeran et al., 2011). Denotation of the genomic sequence of hTERT and elucidation of its gene organization (Cong et al., 1999; Horikawa et al., 1999; Takakura et al., 1999; Wick et al.,

2003) has led to the characterization of hTERT as the limited factor for telomerase activity. Recent developments have paved the way for various cancer-specific diagnostic and therapeutic approaches as well. Telomerase-specific replicative adenovirus (Telomelysis, OBP-301) has been developed as an oncolytic virus that replicates specifically in cancer cells and causes cell death (as reviewed by Kyo et al., 2008). The hypothesis establishing hTERT regulation as a key mechanism in cell immortalization, neoplasms, and the progression of cancers seems solid, however, considerably more research is needed to evolve and elucidate more concrete regulatory mechanisms.

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Highlights

- Telomerase is regulated by the *hTERT* gene.
- The *hTERT* gene is controlled by genetic and epigenetic factors.
- >*hTERT* gene control is important in cancer and aging.

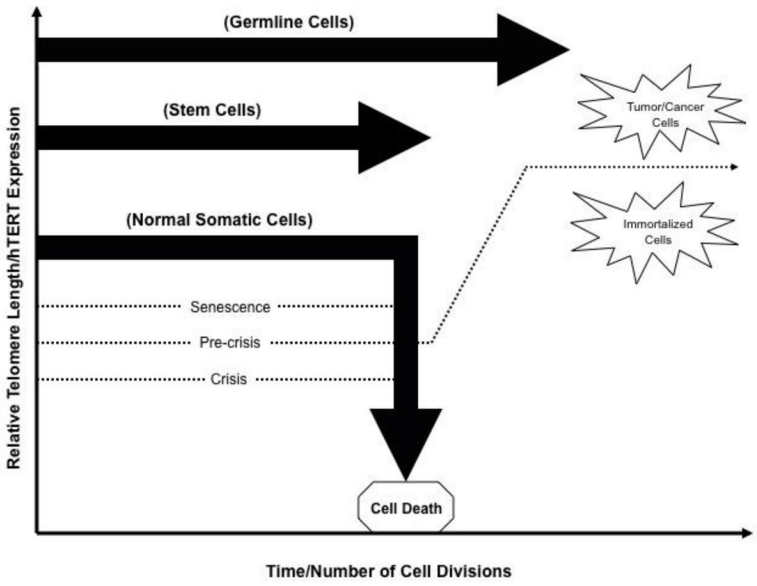

Figure 1. hTERT expression and telomere length during differentiation, immortalization, and tumorigenesis

Diagram depicting the relative changes in telomere length and *hTERT* expression during differentiation, immortalization, and tumorigenesis. As indicated, germline cells exhibit relatively high levels of *hTERT* as well as lengthened telomeres, independently of time and the number of cellular divisions. In stem cells, the telomere lengths and expression of *hTERT* is slightly lower than that of germline cells, however, they do follow the same trend of independence relative to time and/or the number of cellular divisions. During the division of somatic cells, telomere length and *hTERT* expression generally decrease in both a time-dependent and division-dependent manner. Telomere lengths are shortened with each cellular division until senescence occurs followed by a crisis period then apoptosis/cellular death. Activation of telomerase at the pre-crisis stage allows for somatic cells to escape crisis and become immortalized. This occurrence is characteristic in approximately 90% of human cancers (as reviewed by Kyo et al., 2008).

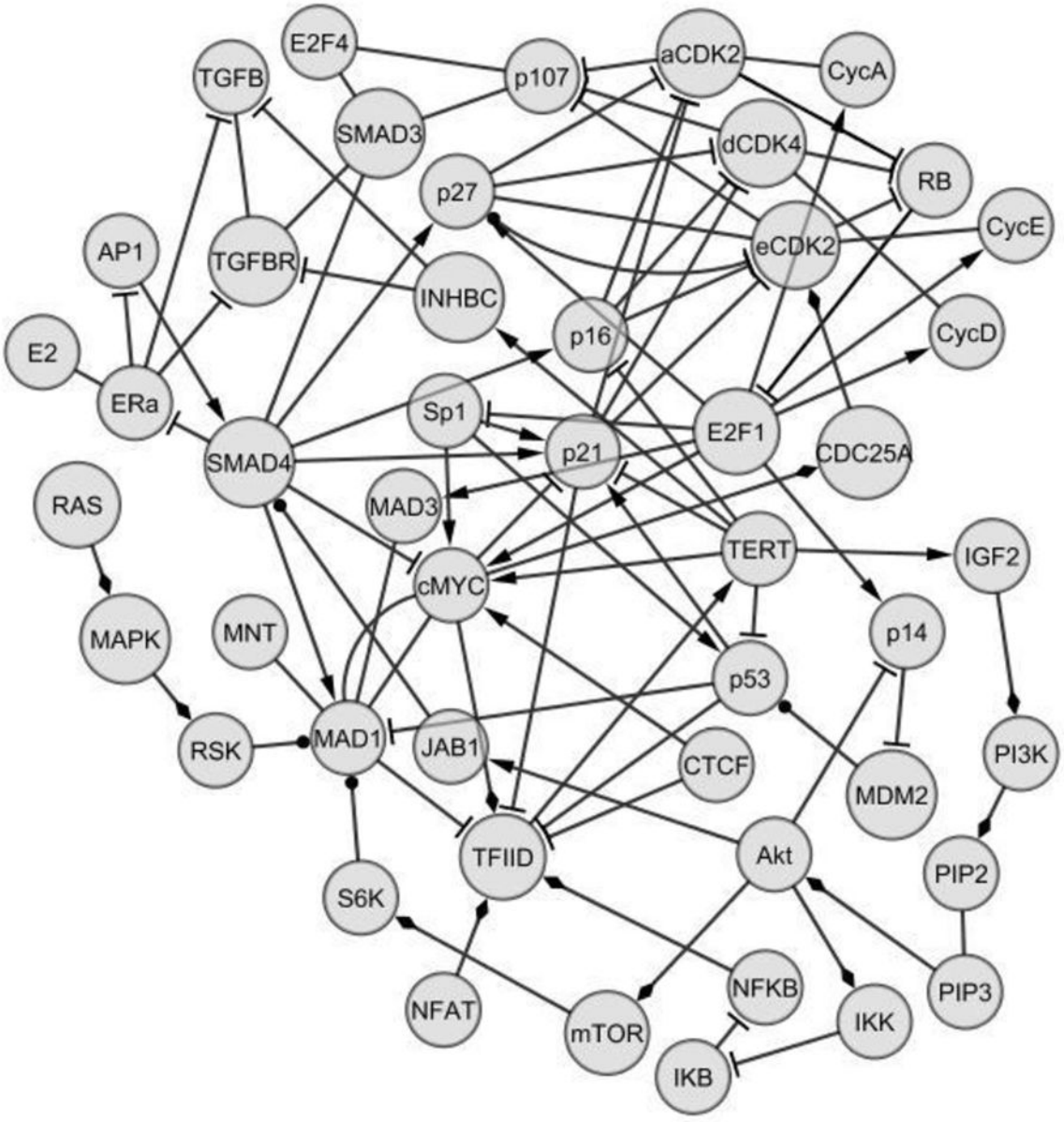

Figure 2. Partial hTERT transcription regulation interactome

The principal protein-protein interactions that regulate *hTERT* involve cross-regulation between *hTERT* and the cell cycle, carried out largely by cyclin/cdk complexes and transcription factors that bind to the *hTERT* promoter. The cyclic nature of the cell cycle is reflected in the dual role of E2F-1 as both repressor and activator. Major regulatory pathways exert either repressive, as in the case of the TGF-b pathway, or activating influence on both the cell cycle and *hTERT* expression. The PI3K/Akt, NF-kB and MAP kinase pathways all activate. In addition, estrogen (E2) bound to estrogen receptor ERa blocks the repressive activity of the TGF-b pathway, as does cyclin/cdk phosphorylation of p107. In a positive feedback loop, expression of *hTERT* activates the PI3K/Akt pathway, which activates the cell cycle by MAD1 and p53 degradation, activates the NF-kB pathway and potentially blocks the TGF-b pathway.

Arrow shape	Meaning
Delta	Increase, transcribe, synergy or assemble
Diamond	Activate
T	Decrease or block
Ball	Degrade

Table 1

Characterization of pathways and constituent and other factors in combined regulation of hTERT transcription.

Factor (hTERT binding sites)	Primary role	References
Pathway – Cell Cycle		
CDK2	Activator	(McArthur et al. 2002)
CDK4	Activator	(Leng et al. 2002)
Cyclin D	Activator	(Leng et al. 2002)
Cyclin E	Activator	(McArthur et al. 2002)
p21	Repressor	(Henderson et al. 2000; Lai et al. 2007; Harper et al. 1993)
p53	Repressor	(Lai et al. 2007; Shats et al. 2004; Chun and Jin 2003)
p27	Mostly repressor	(Ray et al. 2009)
Rb	Mostly repressor	(Wang et al. 2005)
E2F1 (2)	Mostly repressor	(Stanelle et al. 2002)
p14 (p19)	Repressor	(Moore et al. 2003; Guo et al. 2008; Wang et al. 2006)
MDM2	Activator	(Moore et al. 2003; Wang et al. 2006)
p16	Repressor	(Henderson et al. 2000)
Pathway – TGF-β		
TGF-β	Repressor	(Wu et al. 2009)
TGF-βR1	Repressor	(Eickelberg et al. 2002)
TGF-βR2	Repressor	(Eickelberg et al. 2002)
SMAD3	Repressor	(Chen et al. 2002)
SMAD4	Repressor	(Wu et al. 2003; Ren et al. 2009)
E2F4	Repressor	(Chen et al. 2002)
E2F5	Repressor	(Chen et al. 2002)
Pathway – PI3K/Akt		
PI3K	Activator	(Zhu et al. 2008; Bai et al. 2009
Akt	Activator	(Bai et al. 2009; Chou et al. 2009)
PTEN	Repressor	(Feng et al. 2007; Zhou et al. 2006; Phuong et al. 2011)
mTOR	Activator	(Zhu et al. 2008)
IKK	Activator	(Bai et al. 2009)
hTERT	Activator	(Lai et al. 2007; Perrault et al. 2005; Takano et al. 2008)
Pathway – NF-κB (canonical)		
NF-κB (p50) (1)	Activator	(Bai et al. 2009)
NF-κB (p65) (1)	Activator	(Bai et al. 2009)
IKK	Activator	(Bai et al. 2009; Kamata et al. 2002)
IκB	Repressor	(Bai et al. 2009)
Pathway – NF-κB (non-canonical)		
NIK	Activator	(Bai et al. 2009; Varfolomeev et al. 2007)
IKK	Activator	(Bai et al. 2009)

Factor (hTERT binding sites)	Primary role	References
NF-κB (p100) (1)	Activator	(Bai et al. 2009; Varfolomeev et al. 2007)
NF-κB (RelB) (1)	Activator	(Bai et al. 2009; Varfolomeev et al. 2007)
Pathway – MAPK		
RAS	Activator	(Zhu et al. 2008)
MEK	Activator	(Zhu et al. 2008)
ERK	Activator	(Wang et al. 2008)
Pathway – ErbB		
EGF	Activator	(Wang et al. 2008)
EGFR	Activator	(Wang et al. 2008)
HER2/neu	Activator	(Hsu et al. 2008)
PI3K	Activator	(Zhu et al. 2008)
Pathway – TGF-β and Cell Cycle (a link between activation and repression pathways)		
p107	Repressor	(Chen et al. 2002; Beijersbergen et al. 1994)
Pathway – TGF-β and PI3K/Akt (a link between activation and repression pathways)		
Jab1	Activator	(Wan et al. 2002; Tomoda et al. 2002; Oh et al. 2006)
Pathway – Cell Cycle, TGF-β, PI3K/Akt and MAPK		
c-MYC (2)	Activator	(Chen et al. 2002; Casillas et al. 2003)
MAD1 (2)	Repressor	(Zhu et al. 2008; Casillas et al. 2003)
Other		
AP2 (17)	Activator	(Kyo et al. 2008)
AP4 (9)	Activator	(Jung et al. 2008; Jung and Hermeking 2009)
BRCA1	Repressor	(Li et al. 2002)
CCAC (1)	Activator	(Wick et al. 1999)
c-Ets-2 (2)	Activator	(Kyo et al. 2008)
c-IAPI	Activator	(Zhu et al. 2008)
c-Myb (2)	Activator	(Quintana et al. 2011; Drabsch et al. 2010)
CREB/ATF (1)	Activator	(Cong et al. 1999)
CTCF (2)	Mostly repressor	(Renaud et al. 2007)
AP1	Mixed	(Cherlet and Murphy 2007)
ERα (2)	Activator	(Tee et al. 2004)
ERβ (2)	Mostly activator	(Tee et al. 2004)
IK2 (6)	Activator	(Cong et al. 1999)
INHBC	Activator	(Perrault et al. 2005)
MAD3 (2)	Repressor	(Jiang et al. 2008)
MAZ (3)	Activator	(Song et al. 1998; Song et al. 2003)
Menin (2)	Repressor	(Imachi et al. 2010; Suphapeetiporn et al. 2002)
MNT (2)	Repressor	(Hooker and Hurlin 2006)
MyoD (3)	Mixed	(Jin et al. 2011)
MZF-2 (4)	Repressor	(Fujimoto et al. 2000; Ogawa et al. 2003)

Factor (hTERT binding sites)	Primary role	References
NF1 (9)	Activator	(Chikhirzhina et al. 2008)
NFAT (5)	Activator	(Chebel et al., 2009)
NF-E2 (1)	Activator	(Goerttler et al. 2005)
PITX1	Repressor	(Qi et al. 2011)
Sp1 (14)	Mostly activator	(Parisi et al. 2007)
USF1/USF2 (2)	Mixed	(Goueli and Janknecht 2003)
WT1 (1)	Mostly repressor	(Sitaram et al. 2010)