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Complex regulatory mechanisms of telomerase activity in normal and cancer cells: How can we apply them for cancer therapy?

Satoru Kyo*,[1] and Masaki Inoue[1]

[1]*Department of Obstetrics and Gynecology, Kanazawa University, School of Medicine, Japan*

Telomerase activation is observed in almost 90% of human cancers but not in normal tissues of somatic origin and thus is a critical step for multistep carcinogenesis. A more thorough understanding of telomerase regulation may provide not only a molecular basis of cancer progression but also as a way to manipulate telomerase activity as a potential therapeutic modality. Recent progress in studies on telomerase regulation has shown that telomerase activation is achieved at various steps, including transcriptional and post-transcriptional levels of the telomerase reverse transcriptase (hTERT) gene. Although a number of potentially important mechanisms of telomerase activation have been proposed, none of the current models can fully explain tumor-specific activation of telomerase, suggesting a need for further extensive analysis. This review includes a summary of recent works on telomerase regulation and a discussion of how we can overcome this situation.
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Introduction

The maintenance of telomeres, specialized nucleoprotein structures at the ends of chromosome, is essential for chromosome stability (Blackburn, 1991). Without new synthesis, telomeres undergo progressive shortening with each cell division (Watson, 1972), and critically short telomeres under threshold lengths trigger either replicative senescence or apoptosis over a considerable number of cell divisions (Allsopp *et al*., 1992; Harley *et al*., 1990). Telomerase is a ribonucleoprotein complex that extends and maintains the telomeres, and activation of this enzyme is therefore required for cells to overcome replicative senescence and obtain the ability to divide without limits (Counter *et al*., 1992; Greider and Blackburn, 1989). This concept was supported by findings that telomerase activity is observed in the vast majority of cancers or cancer cell lines but not in most normal tissues (Kim *et al*., 1994; Shay and Bacchetti, 1997). Studies of the telomerase enzyme complex have revealed the presence of two major subunits contributing to enzymatic activity: a structural RNA component (hTER) that contains a template region that binds the TTAGGG repeats in telomeres (Feng *et al*., 1995) and a catalytic subunit with reverse transcriptase activity (hTERT) (Meyerson *et al*., 1997; Nakamura *et al*., 1997). Purified hTERT mixed with hTR is sufficient to reconstitute telomerase activity *in vitro* (Masutomi *et al*., 2000). While hTER is constitutively present in normal and cancer cells, expression of hTERT is almost exclusively limited to cancer cells (Meyerson *et al*., 1997; Nakamura *et al*., 1997). Introduction of the hTERT gene into telomerase-negative normal cells is sufficient to induce telomerase activity and to immortalize cells that can be propagated to telomere-based replicative senescence (Bodnar *et al*., 1998; Nakayama *et al*., 1998; Weinrich *et al*., 1997). Telomerase activity and hTERT mRNA expression are tightly associated in human cancers (Takakura *et al*., 1998). *In vitro* transformation of telomerase-negative normal cells using defined genetic elements almost always requires hTERT expression (Hahn *et al*., 1999). These findings indicate that hTERT expression is a rate-limiting step in telomerase activity and carcinogenesis.

Elucidation of the mechanisms governing telomerase activation will likely have wide-ranging effects on the study and treatment of cancers. However, the precise molecular mechanisms of telomerase activation and hTERT expression in cancers remain largely unclear, despite the extensive efforts of a number of research groups attempting to dissect such mechanisms. In this review, these works will be summarized and followed by a discussion of the potential applications of this knowledge to cancer therapy.

When is telomerase activated during carcinogenesis?

It is not fully resolved when telomerase is activated during carcinogenesis. Studies of clinical samples have revealed telomerase activation not only in cancers but also in some types of premalignant lesions, such as cervical intraepithelial neoplasia (Kyo *et al*., 1996; Snijders *et al*., 1998), prostate intraepithelial neoplasia (Zhang *et al*., 1998), and even some benign lesions, suggesting that telomerase activation is an early event in carcinogenesis. However, in general, levels of telomerase activity in these lesions are low compared to those in cancers (Kyo *et al*., 1998), creating some doubt as to the biological significance of this activity. There are some controversies regarding the correlations between the

*Correspondence: S Kyo; E-mail: satoruky@med.kanazawa-u.ac.jp

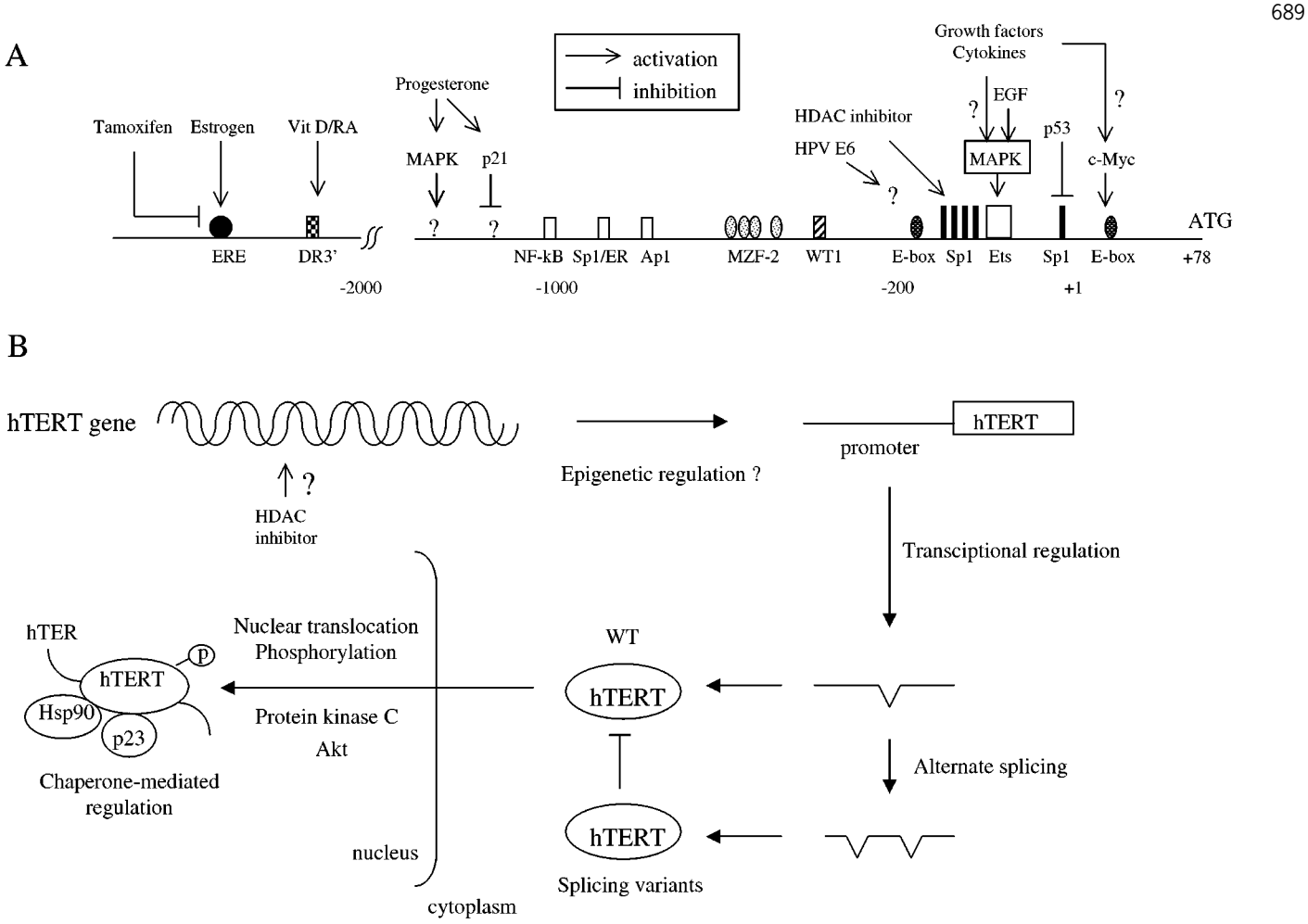

Figure 1 Schematic representation of the possible regulatory mechanisms of telomerase activity. (**a**) Potential cis-acting elements in the hTERT promoter as well as factors that interact with them are shown. The +1 indicates the start site of transcription, determined by CapSite Hunting method (Takakura *et al*., 1999). ERE: estrogen responsive element. DR3′: degenerated vitamin D3 receptor/retinoid X receptor binding site. WT1: Wilms' tumor 1 tumor suppressor gene product. (**b**) Possible regulatory mechanisms of telomerase activity at various steps

clinico-pathological characteristics of cancers and telomerase activity. Some studies showed increased frequency or levels of telomerase activity in cancers with advanced stages or metastatic phenotypes (Hiyama *et al*., 1995a, 1996; Clark *et al*., 1997), indicating that telomerase activation is a rather late event in cancer progression, while others have failed to observe such correlations. There is thus confusion regarding the points at which telomerase is activated during carcinogenesis.

The role and timing of telomerase activation in carcinogenesis has been well analysed by TERC knockout mouse studies. mTERC null mice exhibit significant telomere erosions, an age- and generation-dependent increase in cytogenic abnormalities, providing evidence that telomere dysfunction with critically short telomeres causes genomic instability (Rudolph *et al*., 1999). This concept is further supported by studies of mTERC −/− p53−/− double-knockout mice (Chin *et al*., 1999). These mouse cells show high levels of genomic instability exemplified by the increased formation of dicentric chromosomes and increased susceptibility to transformation by oncogenes. These mice exhibit significant decreases in tumor latency and overall survival. Thus, in the absence of genome checkpoint functions, telomere dysfunction accelerates genomic instability, which facilitates cancer initiation. According to this concept, it is possible that genomic instability caused by telomere dysfunction occurs in the early stages of carcinogenesis, when telomerase has not yet been activated. During subsequent progression, telomeres in these initiated cells undergo further progressive shortening, generating rampant chromosomal instability, which threatens the survival of these cells. Therefore, telomerase activation necessarily occurs at this stage to stabilize the genome and confer unlimited proliferative capacity upon the emerging and evolving cancer cell.

Telomerase activity in normal cells

Not only cancer cells, but some types of normal cells express telomerase activity, including hematopoietic

progenitor cells, intestinal crypt cells, endometrial cells and basal layer cells of skin and cervical keratinocytes (Counter *et al*., 1995; Harle-Bachor and Boukamp, 1996; Hiyama *et al*., 1995b; Kyo *et al*., 1997; Yasumoto *et al*., 1996). The common feature of these telomerase-positive normal cells is their highly regenerative capacity. These cells exhibit continuous or cyclic regeneration throughout human life. Telomerase activity in these cells is tightly associated with cellular proliferation. In hematopoietic progenitor cells, telomerase activity is weakly detected in the primitive stem cells. However, once these cells undergo *ex vivo* expansion under mitogenic stimulation, telomerase activity is dramatically increased (Hiyama *et al*., 1995b). Human endometrium is a unique tissue that undergoes a cyclic pattern of proliferation, secretory activity and breakdown over an approximately 28-day period. Epithelial glandular cells are the source of telomerase activity in endometrium, and telomerase activity in these cells cyclically changes in a menstrual phase-dependent manner, associated with cellular proliferation (Tanaka *et al*., 1998). Notably, telomerase activity in proliferative phase endometrium is unexpectedly strong, comparable to the activity observed in endometrial cancer cells (Kyo *et al*., 1997).

The physiological roles of this proliferation-dependent telomerase regulation in normal cells remain unclear. During *ex vivo* expansion of hematopoietic cells, telomere length decreases despite the presence of telomerase activity, suggesting that telomerase activity alone is insufficient to completely prevent telomere shortening in these cells (Engelhardt *et al*., 1997). However, the rate of base pair loss per population doubling decreases in the presence of telomerase activity during expansion, indicating that telomerase activation may slow down the rate of telomere erosion in these cells. Telomerase activation may thus be an adaptive response to protect excessive telomere loss and possibly may help to extend the proliferative life span of highly regenerative cells.

Which cells are the sources of telomerase activity in these regenerative tissues? Is telomerase activity present in a small subset of cells with stem cell-like characters or in wide-range of more mature cells? Immunohistochemical analysis of human endometrium using hTERT antibody reveals that hTERT is broadly expressed in epithelial glandular cells, preferentially in the proliferative phase (Kyo *et al*., unpublished data). These findings support the concept that maturated proliferating cells rather than a small subset of stem-like cells are responsible for telomerase activity in endometrium. In contrast, telomerase activity in hematopoietic cells, is detected only in primitive stem cells, such as $CD34^+CD38^+$ cells or $CD34^+CD^-$ cells (Hiyama *et al*., 1995b). Similarly, telomerase activity in normal human keratinocytes of uterine cervix, is detected in a small subset that expresses predominantly integrin $\beta 1$ and EGFR (Kunimura *et al*., 1998). The spectrum of telomerase-positive normal cells may therefore differ according to tissue types.

The molecular mechanisms of this proliferation-dependent telomerase regulation remain unclear. There are several lines of evidence suggesting that factors regulating cell proliferation directly or indirectly also regulates telomerase. This is discussed in the following sections. Telomerase regulation during the cell cycle has been an area of contention. Some studies have suggested cell cycle-dependent regulation of telomerase occurs during S phase (Zhu *et al*., 1996). However, subsequent analysis revealed that telomerase activity does not change significantly during progression through the stages of the cell cycle, but is lost when cells exit the cell cycle and enter G0 phase (Holt *et al*., 1997).

How is telomerase regulated?

Of all the constituents of the telomerase complex, hTERT appears to be the key molecule involved in telomerase regulation. Regulation of hTERT expression is achieved at various levels, including transcriptional and post-transcriptional (Figure 1).

(1) Transcriptional regulation of hTERT

Transcriptional regulation of hTERT is thought to be the major mechanism of telomerase regulation. The hTERT promoter is a GC-rich, TATA-less promoter (Cong *et al*., 1999; Horikawa *et al*., 1999; Takakura *et al*., 1999). Deletion analyses in reporter assays show that the proximal region of the hTERT promoter is responsible for most of the transcriptional activity. Indeed, the 200-bp proximal region, designated as the hTERT core promoter, contains the basal transcriptional activity of hTERT (Takakura *et al*., 1999). In the core promoter, multiple E-boxes and Sp1 binding sites are located. c-Myc binds to these E-boxes through heterodimer formation with Max proteins and activates transcription of hTERT (Wu *et al*., 1999; Greenberg *et al*., 1999). This is a direct effect of c-*myc* that does not require *de novo* protein synthesis. Mad proteins are antagonists of c-Myc and switching from Myc/Max binding to Mad/Max binding decreases promoter activity of hTERT (Gunes *et al*., 2000; Kyo *et al*., 2000; Oh *et al*., 2000; Xu *et al*., 2001). Sp1 is also a key molecule that binds to GC-rich sites on the core promoter and activates hTERT transcription (Kyo *et al*., 2000). Cooperative action of c-Myc and Sp1 is required for full activation of hTERT promoter. Overexpression of c-Myc is frequently observed in a wide variety of tumor types, and usually results from chromosome translocation involving the c-Myc genes in addition to gene amplification (Alitalo *et al*., 1987). During multistep carcinogenesis using fibroblast lineages transfected with SV40 LT, expression levels of c-Myc and Sp1 correlate with the levels of telomerase activity in different stages of transformation (Kyo *et al*., 2000). Thus, up-regulation of these critical transcription factors may, at least in part, be involved in telomerase activation during carcinogenesis. Can

cancer-specific telomerase activation be explained only by c-Myc and Sp1 functions? Although overexpression of the c-Myc gene is observed in a significant proportion of tumors, some tumors lack Myc overexpression despite the presence of telomerase activity. Transfer of a normal chromosome 3 into the breast cancer cells represses hTERT expression without affecting c-Myc or Mad levels, or expression of c-Myc target genes (Ducrest *et al*., 2001). Sp1 protein is a ubiquitous factor, which is abundant in some types of normal cells, but such cells do not always have high telomerase activity. Thus, up-regulation of these factors is not sufficient to explain cancer-specific telomerase activation.

Are any other transcription factors involved in telomerase activation? There are several putative binding sites for known transcription factors, including NF-κB and Ap1, in upstream sequences of the hTERT promoter, and mutations in these sites decrease promoter activity, indicating that these factors may be involved in transcriptional activation (Kyo *et al*., unpublished data). However, these factors are not cancer-specific and do not contribute much to cancer-specific hTERT expression.

Several lines of evidence suggest that hTERT expression behaves like a recessive trait. Hybrids between immortal cells with and without telomerase activity, or transfers of single chromosomes or parts of chromosomes from normal diploid cells to breast cancer cells, generate hybrid clones with repressed telomerase, which then subsequently exhibit growth arrest or senescence (Bryan *et al*., 1995; Cuthbert *et al*., 1999). However, some hybrid clones can continue to proliferate with long telomeres. These results suggest that normal cells or telomerase-negative immortal cells contain a gene or genes functioning as telomerase repressors, and the latter maintains telomere length through a dominant mechanism other than telomerase reactivation. Therefore, one hypothesis contends that telomerase repressors may exist in normal cells, which contribute to the silencing of telomerase but their function or expression is lost in tumors, leading to the reactivation of telomerase. Identification of the transcriptional repressors interacting with the hTERT promoter is essential to more fully understand the regulation of telomerase, but few telomerase repressors have been reported so far. In reporter assays, we identified a 400-bp silencer region between -776 and -378 of the hTERT promoter (the transcription start site is numbered as $+1$) (Fujimoto *et al*., 2000). The inhibitory effects of this silencer are enhanced by cellular differentiation. There are multiple binding sites for myeloid-specific zinc finger protein 2 (MZF-2) within this region, and specific binding was confirmed by gel shift assays, and overexpression of MZF-2 represses promoter activity, while mutation of these sites activates it. MZF-2 may thus be an effector of negative regulation of hTERT (Fujimoto *et al*., 2000). However, expression of MZF-2 is broadly observed not only in normal cells, but also in cancer cells, and thus unlikely to represent a specific telomerase repressor in normal cells. Another candidates for hTERT repression is the Wilms' tumor 1 tumor suppressor gene product (WT1), isolated using an expression cloning approach using cDNA library prepared from normal human kidney tissues (Oh *et al*., 1999). WT1 specifically binds to GCGCGGGCG at -281 (numbering based on the transcription start site determined by CapSite Hunting method (Takakura *et al*., 1999)), and mutations in this site dramatically induce reporter gene expression in cells known to express WT1. Overexpression of WT1 significantly inhibits telomerases, suggesting that WT1 may be a transcriptional repressor of hTERT. However, WT 1 gene is expressed in limited cell types, such as the kidney, gonad and spleen, and 293 kidney cells exhibit hTERT mRNA expression despite the presence of endogeneous WT1 protein. The role of WT1 as a repressor therefore seems to be limited.

(2) Alternate splicing of hTERT

Alternate splicing of hTERT transcripts appears to have at least some role in telomerase regulation. The hTERT transcript has at least six alternate splicing sites (four insertion sites and two deletion sites), and variants containing both or either of the deletion sites are present during development as well as in a panel of cancer cell lines (Ulaner *et al*., 1998). One deletion (β site) and all four insertions cause premature translation terminations, whereas the other deletion (α site) is 36 bp and lies within a reverse transcriptase (RT) motif. However, introduction of splicing variants that contain the α, β or both α and β deletion sites fail to reconstitute telomerase activity in telomerase-negative cells (Yi *et al*., 2000), suggesting that alternate splicing may be one mechanism of telomerase regulation. Interestingly, splicing variants that lack the α site function as dominant-negative inhibitors of telomerase causing telomere shortening and eventually cell death (Colgin *et al*., 2000; Yi *et al*., 2000). In an analysis of clinical samples, tumors expressing splicing variants of hTERT have been shown to lack telomerase activity (Ulaner *et al*., 2000). However, some normal tissues with hTERT transcripts containing both α and β lack telomerase activity (Ulaner *et al*., 2000), suggesting that there are further mechanisms for suppressing telomerase activity downstream of hTERT transcription and mRNA splicing, and these mechanisms have been lost during neoplastic transformation.

(3) Epigenetic regulation of hTERT

DNA methylation of promoters for certain genes, such as cell cycle regulators and tumor suppressor genes, is responsible for loss of expression of these genes in some cancers. The presence of a large CpG island with dense GC-rich content in the hTERT promoter suggests that DNA methylation and chromatin structure may play a role in the regulation of hTERT expression. However, a generalized pattern of site-specific or region-specific methylation correlating with

expression of the hTERT gene has not been identified (Dessain *et al*., 2000; Devereux *et al*., 1999), suggesting that this type of regulation is not a major mechanism involved in telomerase regulation.

Posttranscriptional modifications of histones have been implicated in the physiological control of chromatin structure (Stein *et al*., 2000). Acetylation of the lysine-residue of nucleosomal histones is assumed to lead to local chromatin decondensation, resulting in increasing accessibility of particular DNA regions for RNA polymerase complexes. Histone acetylation is a dynamic process catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC). Several transcription factors, such as Mad, can repress transcription by recruiting HDACs to specific sites in certain promoters (Hassig *et al*., 1997; Heinzel *et al*., 1997; Laherty *et al*., 1997). HDAC1 also mediates transcriptional repression via the Sp1 binding sites (Doetzlhofer *et al*., 1999). Given that the core promoter of hTERT contains both E-box elements that bind to Mad and multiple Sp1 sites, the possibility exists that histone acetylation is involved in transcriptional regulation of hTERT. As expected, HDAC inhibitors can induce hTERT transcription in a variety of normal cells (Cong and Bacchetti, 2000; Takakura *et al*., 2000). The responsive element of the hTERT promoter for this regulation is localized to the proximal 200-bp core promoter region including E-boxes and Sp1 sites (Takakura *et al*., 2001). HDAC inhibitor-induced hTERT activation requires intact Sp1 sites but not E-boxes in the promoter, and overexpression of dominant negative form of Sp1 abrogates this regulation (Takakura *et al*., 2001). Thus, Sp1, but not Mad, plays a central role in this regulation. Conflicting data, however, has been published. Differentiation of leukemia cells involves switching of Myc/Max to Mad/Max binding to the E-boxes on hTERT promoter, associated with decreased histone acetylation of the hTERT promoter (Xu *et al*., 2001). Treatment with HDAC inhibitors facilitates histone acetylation of the hTERT promoter and attenuates differentiation-induced repression of hTERT transcription. Histone acetylation and deacetylation may therefore mediate Myc/Max and Mad/Max regulation of the hTERT promoter via E-boxes. These studies have been based, however, on regulation analyses using cancer cell lines, and the role of these mechanisms in tumor-specific hTERT activation remains unclear. Although HDAC inhibitors can easily activate hTERT transcription in a variety of normal cells, direct evidence has not yet shown that histone deacethylation of the hTERT promoter in normal cells is attenuated during carcinogenesis.

(4) Phosphorylation of hTERT protein

Phosphorylation of hTERT protein may be one mechanism of hTERT activation. Telomerase activity in human breast cancer cells is markedly inhibited by the treatment with protein phosphatase 2A (Li *et al*., 1997). Some protein kinases, such as Atk kinase and protein kinase C have been reported to mediate phosphorylation of hTERT protein, leading to telomerase activation (Kang *et al*., 1999; Li *et al*., 1998). A potentially interesting mechanism of telomerase regulation through hTERT phosphorylation linked to nuclear localization of the emzyme has recently been reported in human T-lymphocytes. Induction of telomerase in resting CD^{4+} T-cells by anti-CD3 stimulation does not require net hTERT protein increase. During this CD^{4+} T cell activation, hTERT is phosphorylated and translocated from the cytoplasm to the nucleus (Liu *et al*., 2001). Thus, nuclear translocation of telomerase from a presumably non-functional cytosolic location to a physiologically relevant nuclear compartment may be one regulatory mechanism of telomerase function in cells. These studies provide evidence for novel control mechanisms of telomerase activity, independent of hTERT protein levels.

(5) Chaperone-mediated regulation

The Hsp90 chaperone complex, including Hsp90, Hsp70 and p23, is functionally associated with telomerase (Holt *et al*., 1999). The Hsp90 chaperone complex is known to facilitate the folding of several reverse transcriptases. In an *in vivo* reconstitution system for telomerase activity, the addition of purified components of the Hsp90 chaperone complex to extracts from cells with weak telomerase activity significantly increased telomerase activity (Akalin *et al*., 2001). A significant portion of telomerase may therefore remain unfolded or unassembled in cells with absent or weak telomerase activity. The addition of the Hsp90 complex would then facilitate telomerase assembly, allowing for enhanced telomerase activity. Interestingly, expression of this chaperone complex is up-regulated during malignant transformation or in advanced cancers compared to surrounding non-cancerous tissues (Akalin *et al*., 2001), suggesting that up-regulation of the chaperone complex may play roles in the enhanced telomerase activation observed in cancer cells.

Factors and agents that regulate telomerase activity

Most factors capable of regulating telomerase activity are growth-regulatory pathways that facilitate or inhibit cellular proliferation. Since telomerase activity is regulated and linked to cellular proliferation, some cell cycle regulator genes are likely to play important roles in the regulation of telomerase activity through the control of cell proliferation. Nevertheless, some factors directly regulate telomerase, independent of cellular proliferation.

(1) Hormones

Several hormones are involved in telomerase regulation. Regulation by sex steroid hormones has been

extensively analysed. Estrogen activates telomerase in estrogen receptor (ER)-positive cells through the up-regulation of hTERT mRNA expression (Kyo *et al*., 1999). Degenerated estrogen responsive elements (EREs) are located in the hTERT promoter, which directly binds ER and functions as a cis-acting element in response to estrogen stimulation. Estrogen also activates c-Myc expression in ER-positive cells, contributing to transactivation of hTERT. Thus, estrogen directly and indirectly activates telomerase. Tamoxifen is a non-steroidal anti-estrogen with wide use as an adjuvant therapy for breast cancer. In breast cancer cells, tamoxifen antagonizes estrogen action and inhibits telomerase activity (Aldous *et al*., 1999). This inhibitory effect is mediated by ER as the pure ER antagonist, ICI182780, blocks inhibition (Wang *et al*., unpublished data). Although the precise mechanism is unknown, tamoxifen competitively antagonizes estrogen binding to ER and/or binds to ER affecting the conformation of this receptor protein. This leads to inhibition of estrogen-induced telomerase activation. Progesterone usually functions as an anti-estrogen in a variety of target tissues. In progesterone receptor (PR)-positive breast cancer cells, the regulation of telomerase by progesterone is complex. Initially, progesterone activates telomerase through up-regulation of hTERT mRNA expression, brought about by transcriptional activation of the hTERT promoter (Wang *et al*., 2000). The transcription factors involved in this regulation remain elusive, but Ras/MEK/ERK signaling pathways may be important. Later, progesterone exhibits the opposite effects, inhibiting telomerase activity through transcriptional repression of hTERT. This down-regulation involves induction of p21 expression. Over-expression of p21 leads to inhibition of both telomerase activity and hTERT mRNA expression in PR-positive cells. Blockade of p21 expression using an antisense strategy abrogates progesterone-induced inhibition of telomerase. Thus, p21 may be a mediator of this regulation. It is unclear how p21 inhibits telomerase activity, but it is likely attributable to indirect action through cell cycle arrest. The effects of androgens on telomerase activity have been demonstrated in prostate cancer cells. In androgen-sensitive cell lines, telomerase activity is reduced by androgen deprivation, while treatment with testosterone restores high levels of telomerase activity (Soda *et al*., 2000). This effect is not observed in androgen-independent cell lines. Androgen thus activates telomerase in androgen-sensitive cell lines. However it remains unclear whether this effect is direct or not.

(2) Differentiation inducing agents

Some differentiation-inducing agents can repress telomerase activity in a variety of cancer cells, probably through indirect action via induction of cellular differentiation. Nevertheless, there is evidence of direct action on telomerase by vitamin D and retinoic acids. A heterodimer formed of the vitamin D_3 receptor (VDR) and the retinoid X receptor (RXR) binds to a specific response element known as DR3. DR3 consists of two directly repeated pairs of AGGTGA motifs spaced by three nucleotides (DR3), and modulates the expression of VD3-responsive genes as a cis-element. Notably, the sequence 5′-AGTTCATGGAGTTCA-3′ (named DR3′) located at −2530 on the hTERT promoter is similar to DR3 (Takakura *et al*., 1999).

Gel shift analysis have revealed that the heterodimer of VDR and RXR specifically binds to this DR3′ sequence in response to VD_3 and 9-cis-retinoic acid (RA) stimulation (Ikeda *et al*., unpublished data). Treatment of bladder cancer cells with VD_3 and 9-cis-RA leads to a decrease in hTERT promoter activity, which requires intact DR3′ sequences, as well as inhibiting both hTERT mRNA expression and telomerase activity (Ikeda *et al*., unpublished data). These studies suggest that Vitamin D and retinoic acids are potential anti-telomerase agents that directly down-regulate hTERT transcription. These agents have been used as anti-cancer drugs for various cancers, including hematological malignancies. Long-term treatment of cancer cells with these agents leads to telomere shortening and growth inhibition, possibly due to the telomere dysfunction (Ikeda *et al*., unpublished data). These processes outlined above may be novel mechanisms through which these agents exhibit anti-tumor action.

(3) Growth factors

Several growth factors regulate telomerase activity in normal and tumor cells. Most regulation is achieved by indirect actions, but some growth factors may work through direct pathways to regulate telomerase. EGF is a representative growth factor that facilitates proliferation of a variety of cell types. Once EGFR-positive cells are exposed to EGF, telomerase activity is up regulated following activation of hTERT mRNA expression (Maida *et al*., unpublished data). This is a rapid effect, observed within 6 h after treatment. No requirement for *de novo* protein synthesis has been observed, suggesting a direct effect of EGF. There are several lines of evidence that specific signal transduction pathways mediate this regulation. A specific MEK inhibitor of the Ras/MEK/ERK pathway, abrogates EGF-induced activation of hTERT. In addition, transactivation of hTERT by EGF requires a specific promoter element (TTCCTTTCCG) located at −22, a consensus binding motif for Ets proteins, known to be the major target of EGF signaling. These findings suggest that EGF signals utilize the Ras/MEK/ERK pathway to activate hTERT expression. Thus, there may be other signal transduction pathways for telomerase regulation in response to stimulation by various growth factors.

(4) Anticancer agents

Some anticancer drugs modulate telomerase activity. Cisplatin is a representative chemotherapeutic agent that cross-links DNA between guanines. Since telo-

meres are composed of guanine-rich sequences, it is possible that cisplatin impairs the telomere structure, leading to the inhibition of telomerase activity. Several clinical reports have demonstrated that chemotherapy with Cisplatin leads to significant inhibition of telomerase activity in tumors. However, these effects are probably indirect, following cell cycle arrest, necrosis or apoptosis caused by the action of Cisplatin. When testicular cancer cell lines are treated with Cisplatin, telomerase activity is significantly inhibited but both a high concentration and an interval of 20 h following drug removal are required (Burger *et al*., 1997), suggesting an indirect effect of this agent. At present, no anticancer drug used for cancer chemotherapy seems to directly regulate telomerase activity.

(5) Histone deacetylase inhibitors

The role of histone deacetylase (HDAC) inhibitors in telomerase regulation is complex. In a variety of normal cells without telomerase activity, HDAC inhibitors can induce telomerase activity via up-regulation of hTERT mRNA expression (Cong *et al*., 2000; Takakura *et al*., 2001) indicating that histone deacetylation is involved in transcriptional silencing of hTERT in normal cells. However, in telomerase-positive cancer cells, these agents do not alter telomerase activity or appear to repress it in higher concentrations (Takakura *et al*., 2001). The roles of these agents are therefore unclear. These agents are thought to be potentially effective agents for cancer therapy, based on the findings that HDAC inhibitors can induce growth arrest, differentiation, and/or apoptotic cell death in a wide variety of cancer cells (Marks *et al*., 2000). It is therefore important to clarify the role of these agents in telomerase regulation in cancer cells. One possible mechanism for their diverse effects in cancer and normal cells may be that telomerase has already been fully activated through various mechanisms, including through histone acetylation of the hTERT promoter. HDAC inhibitors may therefore be unable to induce further telomerase activity. Alternatively, HDAC inhibitors induce $p21^{WAF1}$ expression in cancer cells probably through histone-acetylation of the promoter (Sowa *et al*., 1999; Richon *et al*., 2000). This may play an important role in the arrest of cell growth or cellular differentiation, leading to indirect inhibition of telomerase activity.

(6) Cell cycle regulators

Some cell cycle regulators are involved in telomerase regulation. Overexpression of p53 effectively represses telomerase activity through transcriptional down-regulation of hTERT in a variety of cancer cell lines (Kanaya *et al*., 2000; Xu *et al*., 2000). This effect is independent of p53-induced apoptosis or induction of the p53 target gene p21 (Kanaya *et al*., 2000). Even though there are two p53 binding motifs outside the core promoter of hTERT, reporter assays reveal that the core promoter is responsible for this regulation, requiring intact Sp1 binding sites (Kanaya *et al*., 2000). p53 inhibits Sp1 binding to the core promoter with the formation of a p53-Sp1 complex (Xu *et al*., 2000). Therefore, protein–protein interaction of p53 with Sp1 may play critical roles in this regulation. In addition, p21, p15 and p16 exert some inhibitory effects on telomerase activity, altough these are probably secondary effects following the induction of cell cycle arrest (Fuxe *et al*., 2000; Kagawa *et al*., 1999; Sawa *et al*., 1999). There are two putative E2F-1-binding sites proximal to the transcriptional start site of the hTERT promoter. Mutation of these sites produce dramatic increases in promoter activity, whereas overexpression of E2F-1 represses it, which requires intact E2F-1-binding sites (Crowe *et al*., 2001). Human cancer cell lines stably overexpressing E2F-1 exhibit decreased hTERT mRNA expression and telomerase activity. These findings suggest that E2F-1 possesses an atypical function as a transcriptional repressor of the hTERT gene in human cells.

(7) Oncogenes

The human papillomavirus E6 oncoprotein activates telomerase (Klingelhutz *et al*., 1996). This effect is cell-type specific, observed in mammary epithelial cells or keratinocytes but not in fibroblasts. It is not fully understood how E6 activates telomerase in a cell-type specific manner. The findings that E6 can activate the c-Myc promoter (Kinoshita *et al*., 1997) or that E6 post-transcriptionally activates c-Myc protein expression (Wang *et al*., 1998) suggest the involvement of c-Myc function in this regulation. However, unexpectedly, c-Myc levels in E6-transduced cells are not well correlated with levels of telomerase activity (Gewin and Galloway, 2001; Oh *et al*., 2001; Veldman *et al*., 2001). Reporter assays reveal that the proximal core promoter is responsible for E6-mediated transactivation (Oh *et al*., 2001; Veldman *et al*., 2001). However, this regulation does not require intact c-Myc binding sites in the hTERT promoter. These findings suggest that myc-dependent transactivation is not essential for it. The role of other oncogenes in telomerase regulation is largely unknown.

How can we control telomerase activity?

Several strategies have been proposed to inhibit telomerase activity in cells. Antisense technologies against hTER and hTERT, ribozymes against hTER, and introduction of a dominant negative form of hTERT are the most powerful approaches to directly target telomerase.

Other approaches include the use of antiestrogens, progesterone, vitamin D and retinoic acid that appear to have their own pathways for telomerase inhibition. These agents are widely used as anticancer approaches for some tumors, including endometrial cancers and hematological malignancies. Signal transduction inhibitors may find additional utility as anti-telomerase

approaches, as some growth factors use these pathways for telomerase regulation. Several clinical trials using signal transduction inhibitors as novel anticancer drugs are ongoing and are obtaining favorable results in some tumor types. Telomerase is a target for hsp90-mediated assembly of the functional enzyme. Therefore, inhibition of hsp90 function by blocking agents would likely inhibit telomerase, but not in a specific manner. Hsp90 blocking agents, such as geldanamycin and its derivatives are touted as anticancer compounds and are currently undergoing clinical trials. However, hsp90 has numerous targets in a given cell, and blanket treatment with hsp90 inhibitors is likely to produce nonspecific effects in both normal and cancer cells.

Despite increasing knowledge regarding agents that inhibit telomerase activity, few studies have reported the long-term effects of these agents. If long-term treatment eventually causes telomere dysfunction, leading to chromosomal instability and cell cycle arrest, these agents can be used as specific telomerase inhibitors. In such cases, they might be better used as adjuvant therapies, in combination with chemotherapy or radiotherapy. For example, cytotoxic agents might be used first to reduce tumor bulk, then telomerase inhibitors used over a longer period to induce tumor cell death, when telomeric length becomes critically shortened.

Application of hTERT promoter for cancer gene therapy

Recent progress in our understanding of the controlling mechanisms of the hTERT gene has prompted the use of the hTERT promoter for vectors for cancer gene therapy. This strategy is based on the expectation that the hTERT promoter can confer strong tumor-specific transgene expression, minimizing toxicity to normal cells. *In vitro* and *in vivo* administration of chimeric vectors in which the hTERT promoter is linked to apoptosis-inducing genes, such as caspase, FADD or Bax, successfully induces apoptosis in cancer cells without damaging normal cells (Gu *et al*., 2000; Komata *et al*., 2001; Koga *et al*., 2000). Notably, the minimal 200-bp core promoter of hTERT is sufficient to effectively induce apoptosis. However, one concern is that the hTERT promoter activity in cancer cells may be insufficient for effective transgene expression, compared to the universal promoters, such as SV40 or CMV promoter. According to *in vitro* reporter assays, the transcriptional activity of hTERT is dependent on cell type. In cancer cells with extremely high levels of telomerase activity, hTERT promoter activity is equivalent to that of the SV40 promoter (Takakura *et al*., 1999). However, in cancer cells with weak telomerase activity, promoter activity is approximately 10–20% of that of SV40 promoter. Although at present little data exists regarding the minimum levels of promoter activity that is required for efficient expression of transgenes to induce apoptosis or inhibit cell growth, the variation in promoter activity according to cell types should be considered when using the hTERT promoter.

Conclusion

Extensive research on the transcriptional regulation of the hTERT promoter identified several factors, including c-Myc and Sp1, which critically regulate promoter activity. However, most factors are not stringently tumor-specific and are also expressed in some normal cells that lack telomerase activity. Thus, tumor-specific hTERT expression cannot be explained simply by these transcription factors. Alternatively, tumor-specificity of hTERT may be explained by unknown 'repressors' that are expressed in normal cells but are lost in cancer cells. Unfortunately, such transcriptional repressors have not yet been identified. A number of chromosome transfer experiments have been conducted to identify these repressors, but no chromosome regions able to inhibit hTERT expression in all cell types have been identified. Epigenetic regulation by DNA methylation and histone acetylation is likewise insufficient to the task of explaining tumor specificity of hTERT expression. Studies on phosphorylation and subcellular localization of hTERT may possess the potential to provide a clearer understanding of telomerase regulation. However, antibodies against hTERT, which are indispensable for such studies, have not been well characterized, and their validity and specificity have not yet been established. Careful evaluation of the results will therefore be required. In summary, despite extensive efforts by a number of groups, the mechanisms of tumor-specific telomerase activation are still not established. Further analyses are required to allow a more complete understanding of the regulation of telomerase activity to emerge. Once obtained, such an understanding will provide a solid foundation for further investigation and manipulation of telomerase activity as a potential therapeutic modality.

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References

Akalin A, Elmore LW, Forsythe HL, Amaker BA, McCollum ED, Nelson PS, Ware JL and Holt SE. (2001). *Cancer Res.,* **61,** 4791–4796.

Aldous WK, Marean AJ, DeHart MJ, Matej LA and Moore KH. (1999). *Cancer,* **85,** 1523–1529.

Allsopp RC, Vaziri H and Patterson C. (1992). *Proc. Natl. Acad. Sci. USA,* **89,** 10114–10118.

Alitalo K, Koskinen P, Makela TP, Saksela K, Sistonen L and Winqvist R. (1987). *Biochim. Biophys Acta.,* **907,** 1–32.

Blackburn EH. (1991). *Nature,* **350,** 569–573.
Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S and Wright WE. (1998). *Science,* **279,** 349–352.
Bryan TM, Englezou A, Gupta J, Bacchetti S and Reddel RR. (1995). *EMBO J.,* **14,** 4240–4248.
Burger AM, Double JA and Newell DR. (1997). *Eur. J. Cancer,* **33,** 638–644.
Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, Greider CW and DePinho RA. (1999). *Cell,* **97,** 527–538.
Clark GM, Osborne CK, Levitt D, Wu F and Kim NW. (1997). *J. Natl. Cancer Inst.,* **89,** 1874–1881.
Colgin LM, Wilkinson C, Englezou A, Kilian A, Robinson MO, and Reddel RR. (2000). *Neoplasia,* **2,** 426–432.
Cong YS and Bacchetti S. (2000). *J. Biol. Chem.,* **275,** 35665–35668.
Cong YS, Wen J and Bacchetti S. (1999). *Hum. Mol. Genet.,* **8,** 137–142.
Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB and Bacchetti S. (1992). *EMBO J.,* **11,** 1921–1929.
Counter CM, Gupta J, Harley CB, Leber B and Bacchetti S. (1995). *Blood,* **85,** 2315–2320.
Crowe DL, Nguyen DC, Tsang KJ and Kyo S. (2001). *Nucleic Acids Res.,* **29,** 2789–2794.
Cuthbert AP, Bond J, Trott DA, Gill S, Broni J, Marriott A, Khoudoli G, Parkinson EK, Cooper CS and Newbold RF. (1999). *J. Natl. Cancer Inst.,* **91,** 37–45.
Dessain SK, Yu H, Reddel RR, Beijersbergen RL and Weinberg RA. (2000). *Cancer Res.,* **60,** 537–541.
Devereux TR, Horikawa I, Anna CH, Annab LA, Afshari CA and Barrett JC. (1999). *Cancer Res.,* **59,** 6087–6090.
Doetzlhofer A, Rotheneder H, Lagger G, Koranda M, Kurtev V, Brosch G, Wintersberger E and Seiser C. (1999). *Mol. Cell. Biol.,* **19,** 5504–5511.
Ducrest AL, Amacker M, Mathieu YD, Cuthbert AP, Trott DA, Newbold RF, Nabholz M and Lingner J. (2001). *Cancer Res.,* **61,** 7594–7602.
Engelhardt M, Kumar R, Albanell J, Pettengell R, Han W and Moore MAS. (1997). *Blood,* **90,** 182–193.
Feng J, Funk WD, Wang SS, Weinrich AAA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, Le S, West MD, Harley CB, Andrew WH, Greider CW and Villeponteau B. (1995). *Science,* **269,** 1236–1241.
Fujimoto K, Kyo S, Takakura M, Kanaya T, Kitagawa Y, Itoh H, Takahashi M and Inoue M. (2000). *Nucleic Acids Res.,* **28,** 2557–2562.
Fuxe J, Akusjarvi G, Goike HM, Roos G, Collins VP and Pettersson RF. (2000). *Cell Growth Differ.,* **11,** 373–384.
Gewin L and Galloway DA. (2001). *J. Virol.,* **75,** 7198–7201.
Greenberg RA, O'Hagan RC, Deng H, Xiao Q, Hann SR, Adams RR, Lichtsteiner S, Chin L, Morin GB and DePinho RA. (1999). *Oncogene,* **18,** 1219–1226.
Greider CW and Blackburn EH. (1989). *Nature,* **337,** 331–337.
Gu J, Kagawa S, Takakura M, Kyo S, Inoue M, Roth JA and Fang B. (2000). *Cancer Res.,* **60,** 5359–5364.
Gunes C, Lichtsteiner S, Vasserot AP and Englert C. (2000). *Cancer Res.,* **60,** 2116–2121.
Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW and Weinberg RA. (1999). *Nature,* **400,** 464–468.
Harle-Bachor C and Boukamp P. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 6476–6481.
Harley CB, Futcher BA and Greider CW. (1990). *Nature,* **345,** 458–460.
Hassig CA, Fleischer TC, Billin AN, Schreiber SL and Ayer DE. (1997). *Cell,* **89,** 341–347.
Heinzel T, Lavinsky RM, Mullen TM, Sonderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo D, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK and Rosenfeld MG. (1997). *Nature,* **387,** 43–48.
Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, Hiyama K and Piatyszek MA. (1996). *J. Natl. Cancer Inst.,* **88,** 116–122.
Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek MA and Shay JW. (1995a). *Nat. Med.,* **1,** 249–255.
Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S and Yamakido M. (1995b). *J. Immunol.,* **155,** 3711–3715.
Holt SE, Aisner D, Baur J, Tesmer VM, Dy M, Ouellette M, Trager JB, Morin GB, Toft DO, Shay JW, Wright WE and White MA. (1999). *Genes Dev.,* **13,** 817–826.
Holt SE, Aisner DL, Shay JW and Wright WE. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 10687–10692.
Horikawa I, Cable PL, Afshari C and Barrett JC. (1999). *Cancer Res.,* **59,** 826–830.
Kagawa S, Fujiwara T, Kadowaki Y, Fukazawa T, Sok-Joo R, Roth JA and Tanaka N. (1999). *Cell Death Differ.,* **6,** 765–772.
Kanaya T, Kyo S, Hamada K, Takakura M, Kitagawa Y, Harada H and Inoue M. (2000). *Clin. Cancer Res.,* **6,** 1239–1247.
Kang SS, Kwon T, Kwon DY and Do SI. (1999). *J. Biol. Chem.,* **274,** 13085–13090.
Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL and Shay JW. (1994). *Science,* **266,** 2011–2015.
Kinoshita T, Shirasawa H, Shino Y, Moriya H, Desbarats L, Eilers M and Shimizu B. (1997). *Virology,* **232,** 53–61.
Klingelhutz AJ, Foster SA and McDougall JK. (1996). *Nature,* **380,** 79–82.
Koga S, Hirohata S, Kondo Y, Komata T, Takakura M, Inoue M, Kyo S and Kondo S. (2000). *Hum. Gene Ther.,* **10,** 1397–1406.
Komata T, Kondo Y, Kanzawa T, Hirohata S, Koga S, Sumiyoshi H, Srinivasula SM, Barna BP, Alnemri ES, Germano IM, Takakura M, Inoue M, Alnemri ES, Shay JW, Kyo S and Kondo S. (2001). *Cancer Res.,* **61,** 5796–5802.
Kunimura C, Kikuchi K, Ahmed N, Shimizu A and Yasumoto S. (1998). *Oncogene,* **17,** 187–197.
Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, Orimo A and Inoue M. (1999). *Cancer Res.,* **59,** 5917–5921.
Kyo S, Takakura M, Kohama T and Inoue M. (1997). Telomerase activity in human endometrium. *Cancer Res.,* **57,** 610–614.
Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, Ariga H and Inoue M. (2000). *Nucleic Acids Res.,* **28,** 669–677.
Kyo S, Takakura M, Tanaka M, Kanaya T and Inoue M. (1998). *Int. J. Cancer,* **79,** 66–70.
Kyo S, Ueno H, Kanaya T and Inoue M. (1996). *Clin. Cancer Res.,* **2,** 2023–2028.
Laherty CD, Yang WM, Sun JM, Davie JR, Seto E and Eisenman RN. (1997). *Cell,* **89,** 349–356.
Li H, Zhao LL, Funder JW and Liu JP. (1997). *J. Biol. Chem.,* **272,** 16729–16732.
Li H, Zhao L, Yang Z, Funder JW and Liu JP. (1998). *J. Biol. Chem.,* **273,** 33436–33442.

Liu K, Hodes RJ and Weng NP. (2001). *J. Immunol.,* **166,** 4826–4830.
Marks PA, Richon VM and Rifkind RA. (2000). *J. Natl. Cancer Inst.,* **92,** 1210–1216.
Masutomi K, Kaneko S, Hayashi N, Yamashita T, Shirota Y, Kobayashi K and Murakami S. (2000). *J. Biol. Chem.,* **275,** 22568–22573.
Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu Q, Bacchetti S, Haber DA and Weinberg RA. (1997). *Cell,* **90,** 785–795.
Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB and Cech TR. (1997). *Science,* **277,** 955–959.
Nakayama J, Tahara H, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Tahara E, Ide T and Ishikawa F. (1998). *Nat. Genet.,* **18,** 65–68.
Oh S, Song YH, Yim J and Kim TK. (1999). *J. Biol. Chem.,* **274,** 37473–37478.
Oh S, Song YH, Yim J and Kim TK. (2000). *Oncogene,* **19,** 1485–1490.
Oh ST, Kyo S and Laimins LA. (2001). *J. Virol.,* **75,** 5559–5566.
Richon VM, Sandhoff TW, Rifkind RA and Marks PA. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 10014–10019.
Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C and DePinho RA. (1999). *Cell,* **96,** 701–712.
Sawa H, Kamada H, Ohshima TA, Noguchi A, Itoh N, Saruta K, Hara M and Saito I. (1999). *J. Neurooncol.,* **42,** 45–57.
Shay JW and Bacchetti S. (1997). *Eur. J. Cancer,* **33,** 787–791.
Snijders PJ, van Duin M, Walboomers JM, Steenbergen RD, Risse EK, Helmerhorst TJ, Verheijen RH and Meijer CJ. (1998). *Cancer Res.,* **58,** 3812–3818.
Soda H, Raymond E, Sharma S, Lawrence R, Davidson K, Oka M, Kohno S, Izbicka E and Von Hoff DD. (2000). *Prostate,* **43,** 161–168.
Sowa Y, Orita T, Minamikawa-Hiranabe S, Mizuno T, Nomura H and Sakai T. (1999). **59,** 4266–4270.
Stein GS, Montecino M, van Wijnen AJ, Stein JL and Lian JB. (2000). *Cancer Res.,* **60,** 2067–2076.
Takakura M, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M and Inoue M. (1999). *Cancer Res.,* **59,** 551–557.
Takakura M, Kyo S, Kanaya T, Tanaka M and Inoue M. (1998). *Cancer Res.,* **58,** 1558–1561.
Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, Maida Y, Tanaka M and Inoue M. (2001). *Nucleic Acids Res.,* **29,** 3006–3011.
Tanaka M, Kyo S, Takakura M, Kanaya T, Sagawa T, Yamashita K, Okada Y, Hiyama E and Inoue M. (1998). *Am. J. Pathol.,* **153,** 1985–1991.
Ulaner GA, Hu JF, Vu TH, Giudice LC and Hoffman AR. (1998). *Cancer Res.,* **58,** 4168–4172.
Ulaner GA, Hu JF, Vu TH, Oruganti H, Giudice LC and Hoffman AR. (2000). *Int. J. Cancer,* **85,** 330–335.
Veldman T, Horikawa I, Barrett JC and Schlegel R. (2001). *J. Virol.,* **75,** 4467–4472.
Wang Z, Kyo S, Takakura M, Tanaka M, Yatabe N, Maida Y, Fujiwara M, Hayakawa J, Ohmichi M, Koike K and Inoue M. (2000). *Cancer Res.,* **60,** 5376–5381.
Wang J, Xie LY, Allan S, Beach D and Hannon GJ. (1998). *Genes Dev.,* **12,** 1769–1774.
Watson JD. (1972). *Nat. New. Biol.,* **239,** 197–201.
Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB and Morin GB. (1997). *Nat. Genet.,* **17,** 498–502.
Wu KJ, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J and Dalla-Favera R. (1999). *Nat. Genet.,* **21,** 220–224.
Xu D, Popov N, Hou M, Wang Q, Bjorkholm M, Gruber A, Menkel AR and Henriksson M. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 3826–3831.
Xu D, Wang Q, Gruber A, Bjorkholm M, Chen Z, Zaid A, Selivanova G, Peterson C, Wiman KG and Pisa P. (2000). *Oncogene,* **19,** 5123–5133.
Yasumoto S, Kunimura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, Ide T and Utakoji T. (1996). *Oncogene,***13,** 433–439.
Yi X, White DM, Aisner D, Baur JA, Wright WE and Shay JW. (2000). *Neoplasia,* **2,** 433–440.
Zhang W, Kapusta LR, Slingerland JM and Klotz LH. (1998). *Cancer Res.,* **58,** 619–621.
Zhu X, Kumar R, Mandal M, Sharma N, Sharma HW, Dhingra U, Sokoloski JA, Hsiao R and Narayanan R. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 6091–6095.