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Telomerase is not an oncogene

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In the decade since the telomere hypothesis of cellular aging was proposed, the two essential genes for human telomerase were cloned and characterized, allowing experimental proof of the causal relationships between telomere loss and replicative senescence, and telomerase activation and immortalization. These relationships were established using a variety of cultured human cell types from both normal and tumor tissues, and were largely confirmed in the telomerase knockout mouse. Taken together, the data provide strong support for the potential utility of telomerase detection and inhibition for cancer, and telomerase activation for degenerative diseases. The specificity of the promoter for the telomerase catalytic gene and the antigenicity of the protein product, hTERT, provide additional strategies for killing telomerase-positive tumor cells. Unfortunately, the strong link between telomerase and cancer has led some to confuse telomerase activation with cancer, and others to overstate the cancer risk of telomerase activation therapies for degenerative diseases. This review clarifies the difference between telomerase, which does not cause growth deregulation, and oncogenes, which do. It also addresses the concept of telomerase repression as a tumor suppressor mechanism early in life, with detrimental tissue degeneration and tumor-promoting consequences late in life. This extended view of the telomere hypothesis helps explain how telomerase inhibition can be therapeutic in cancer patients, while controlled telomerase activation for degenerative diseases may actually reduce, rather than increase, the frequency of age-related tumorigenesis.
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Introduction

Cancer is a complex process involving a multi-faceted evolutionary process occurring at the cellular level within organisms. It is triggered by the second law of thermodynamics (disorder increases in a closed system) operating through mutation and cell selection. Oncology research in the past 30 years has largely focussed on how cancer disrupts the orderly structure of somatic tissues through genetic alterations that cause loss of cell cycle control and abnormal cell–cell, and cell–matrix interactions. However, telomere biology, originally studied by basic geneticists and later biochemists working with plants and invertebrates and totally unaware of a strong connection to oncology (reviewed in Blackburn and Szostak, 1989; Greider, 1996), has emerged as an entirely new field rich in relevance to cancer and other age-related diseases.

The telomere hypothesis of cell aging and immortalization

Telomeres are essential genetic elements at chromosome termini maintained in immortal cells by the enzyme telomerase (Figure 1). Our current understanding of the structure and function of telomeres and telomerase is reviewed elsewhere in this issue. The telomere hypothesis linking telomere loss to replicative senescence and telomerase activation to cell immortalization is reviewed in Figure 2. The major concepts of this hypothesis were proven correct by specific manipulation of telomerase in human cells with the cloned components of the enzyme (Bodnar *et al*., 1998; Feng *et al*., 1995; Hahn *et al*., 1999; Nakamura *et al*., 1997; Vaziri and Benchimol, 1999; Zhang *et al*., 1999). Moreover, it has been largely substantiated by the phenotype of the late generation telomerase knockout mouse (Gonzalez-Suarez *et al*., 2000; Herrera *et al*., 1999; Lee *et al*., 1998; Rudolph *et al*., 1999a), which shares remarkable similarities to the phenotype of the autosomal dominant form of dyskeratosis congenita, a genetic disease caused by a telomerase defect (Vulliamy *et al*., 2001). Telomerase-compromized mice and humans with shortened telomeres (e.g., dyskeratosis congenital) have reduced longevity, chromosome fusions, defects in highly proliferative tissues including impaired regenerative and wound-healing capacity, and an increase in incidence of certain tumors.

Exceptions to simple generalizations will always be found, especially when looking across species or in multiple cell types in varied conditions *in vitro* and *in vivo*. Although the basics of the original telomere hypothesis remain valid, important variations exist. For example, certain highly proliferative human cells are capable of expressing low or transiently high levels of telomerase upon commitment to clonal expansion,

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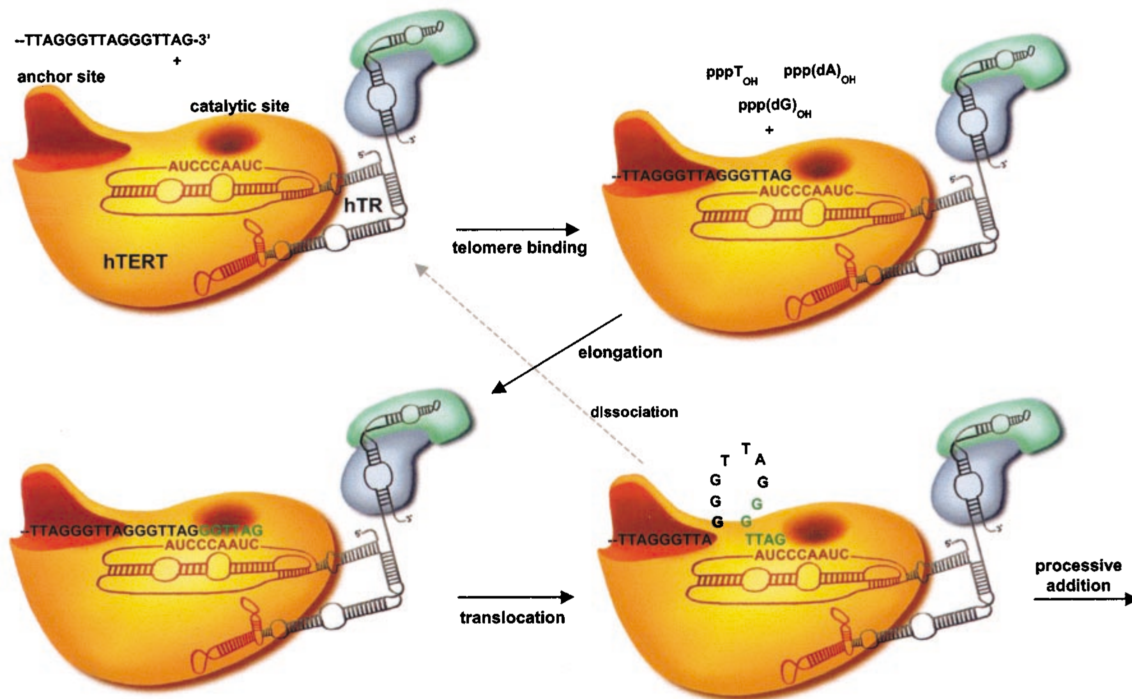

Figure 1 The telomerase mechanism of action. Two protein structures are schematically illustrated: The larger one represents hTERT with shaded regions depicting the telomere substrate 'anchor' site and the catalytic dNTP binding and template alignment site. The smaller structure represents dyskerin and other members of the H+ACA box small nucleolar complex known to be essential for hTR processing and assembly into active telomerase (Mitchell and Collins, 2000). The RNA component hTR is shown folded into a structure based in part on the structure derived from phylogenic analysis of vertebrate TRs (Chen *et al*., 2000), and in part on unique aspects of the hTR sequence. The sequence of the template region of hTR is shown. The processive cycle of telomere binding, elongation through addition of dNTPs, and translocation adds GGTTAG repeats to the 3′ telomeric terminus. The process is interrupted when the extended telomere dissociates from telomerase (dotted line). For simplicity, telomerase is drawn as a single RNP complex and not shown interacting with other telomerase- or telomere-associated proteins

apparently slowing the net rate of telomere loss and conferring an extended, but still finite lifespan to these cells (Bodnar *et al*., 1996; Hiyama *et al*., 1995b; Taylor *et al*., 1996; Usselmann *et al*., 2001; Weng *et al*., 1996, 1997) (Figure 2A, line b). Also, certain transformed human cells capable of indefinite replication in culture are telomerase negative, and maintain unstable telomeres by an alternative lengthening pathway (Bryan *et al*., 1997). Finally, a great deal more is known about telomere associated proteins, the overall telomere structure, and the sensitivity of telomeres to DNA damaging agents. These findings point to additional factors which regulate the rate of telomere loss in the absence of telomerase, the equilibrium telomere length in telomerase positive cells, and provide insights into the consequences of aberrant (or 'uncapped') telomere structures or critical telomere loss (Blackburn, 2001; Goytisolo *et al*., 2000; Griffith *et al*., 1999; Hemann *et al*., 2001; Ranganathan *et al*., 2001; Smith and de Lange, 2000; Smogorzewska *et al*., 2000; von Zglinicki, 2000; Wong *et al*., 2000).

Despite these additions and variations to the telomere hypothesis, there now exists compelling cell and animal data for the potential of killing telomerase-positive cancer cells through telomerase inhibition (Gonzalez-Suarez *et al*., 2000, through therapeutic telomerase vaccines (Minev *et al*., 2000; Nair *et al*., 2000; Vonderheide *et al*., 1999), and through suicide genes driven by the telomerase promoter (Komata *et al*., 2001; Majumdar *et al*., 2001). There is also intriguing cell and animal data for the potential of telomerase activation in degenerative diseases (Funk *et al*., 2000; Jiang *et al*., 1999; Rudolph *et al*., 1999b), supported by the human dyskeratosis congenita genetic evidence (Vulliamy *et al*., 2001). However, human cells in culture and in mice do not capture human physiology, normal human aging is not phenocopied by mutations in telomerase, and man and mouse differ markedly in telomerase regulation, chromosome structure, and the stringency of checkpoint arrest mechanisms. These facts leave uncertain the complete role of telomere loss and telomerase activation in human age-related disease and cancer. Final proof for the medical relevance of these concepts will come with successful human clinical trials using drugs or genes that specifically target telomere or telomerase pathways.

Cancer, growth control and cell immortality

The primary hallmark of tumor cells, common to all cancers and representing one of the first detectable

signs of tissue disorder, is loss of growth control leading to dysplasia and ultimately anaplasia. The two main contributors to this are uncorrected gain-of-function mutations in proto-oncogenes, and loss-of-function mutations in tumor suppressor genes. A second hallmark of tumor cells, common to almost all malignant cancers, is cellular immortality. Whereas normal somatic cells have a finite replicative capacity, some evolving tumor cells appear capable of indefinite replication *in vivo* and *in vitro*. There is strong selective pressure on tumor cells for replicative immortality, since a large number of divisions are exhausted in accumulating the 5–10 independent mutations and clonal expansions typically needed to generate a malignant growth (reviewed in Harley *et al*., 1994).

In human tumors, cell immortalization almost always involves derepression of the gene for the catalytic subunit of telomerase, hTERT (human telomerase reverse transcriptase) (Meyerson *et al*., 1997; Nakamura *et al*., 1997). Transcriptional repression of hTERT early in human development (*in utero*), post-transcriptional processing events, epigenetic changes in the gene, and perhaps other factors which prevent functional telomerase formation, are responsible for the 'mortalization' of normal somatic cells (Cong and Bachetti, 2000; Dessain *et al*., 2000; Poole *et al*., 2001; Ulaner and Giudice, 1997; Ulaner *et al*., 1998; Wright *et al*., 1996). Multiple pathways for stringent repression or controlled regulation of telomerase probably account for the extreme rarity of spontaneous immortalization of normal human cells.

Oncogenes, i.e. mutated or otherwise deregulated proto-oncogenes, are typically regarded as genes that promote the uncontrolled growth of cells. In this context, telomerase is clearly not an oncogene. hTERT and telomerase activity are expressed in normal immortal human embryonic stem cells (Amit *et al*., 2000), in germline cells (Kim *et al*., 1994; Wright *et al*., 1996), and in dozens of somatic cells immortalized by hTERT transduction (Table 1), all without signs of aberrant growth control. Conversely, there are severely growth deregulated, malignant, metastatic tumor cells, such as in neuroblastoma stage IV-S, which are telomerase negative (Hiyama *et al*., 1995a; Reynolds *et al*., 1997). Growth deregulation and cell immortalization are conceptually and physiologically separate processes (Figure 3).

Are there conditions under which telomerase would be considered a proto-oncogene, and the genes controlling cell mortality, i.e. the telomerase repressors, would be considered tumor suppressors? If proto-oncogenes and tumor suppressor genes are restricted to those involved in the active control of cell proliferation, which when altered by gain-of-function or loss-of-function, respectively, give rise to uncontrolled, as opposed to unlimited growth, then the answer is 'no'. But this classical definition of oncogenes and tumor suppressors may be overly narrow. Cellular replicative senescence is generally regarded as a tumor suppressive or protective mechanism. Thus, inactivation of 'growth limiting' genes or deregulated expression of telomerase could increase the frequency of cancer, and hence, by a broader definition linked to cancer risk, the answer would be 'yes'. This argument notwithstanding, telomerase is clearly not a 'classical' oncogene due to the distinction between growth deregulation and immortalization.

To further confound the question of whether telomerase could be considered an oncogene, genetic instability caused by telomere dysfunction resulting from a lack of telomerase is now clearly implicated in tumor initiation in mice. Some evidence also points to a role for this mechanism in humans (Campisi, 1997; Vulliamy *et al*., 2001; Krtolica *et al*., 2001). How can telomerase inhibition be therapeutic in cancer, yet lack of telomerase trigger cancer? The answer to this question is relatively simple: Genetic instability caused by critical telomere loss at cell crisis can contribute to growth deregulating mutations, i.e. transforming events, which may initiate tumor formation. This situation may be particularly acute in rodents, which lack a strong pre-crisis senescence checkpoint. However, in humans, the age-dependent increase in cancer attributable to telomere biology may relate more to loss of normal tissue barriers and diminishing immune function due to senescence, than to genetic instability and mutation.

Progression of telomerase-negative tumors with short telomeres is clearly compromized in telomerase knockout mice (Rudolph *et al*., 2001), as it appears to be in humans. Thus, in patients with malignant telomerase-positive tumors with short telomeres, telomerase inhibition is expected to have a dramatic therapeutic benefit. We do not expect telomerase inhibition therapy to increase the frequency of tumor initiation elsewhere in a human patient, since most normal somatic cells are telomerase negative anyway. The impact of telomerase inhibitors on telomerase competent stem cells should be relatively minor, given (1) their generally long telomeres, (2) normal precrisis checkpoints, and (3) the transient nature of telomerase expression in a subpopulation of stem cells, associated with the initial phases of clonal expansion.

These points help answer the related evolutionary question of how telomerase repression apparently evolved as a tumor suppressor mechanism, whereas telomere dysfunction at senescence, or crisis, apparently contributes to tumor initiation. *Mus musculus,* with extremely long telomeres and lack of stringent telomerase repression in most tissues, does not use a division counting mechanism for tumor suppression (Wright and Shay, 2001) and should not be analysed in this light. In humans, with relatively short telomeres and stringent telomerase repression, it is easy to argue that early in life (when telomeres are not limiting and normal fully functional cells predominate) a telomere-dependent senescence checkpoint in rare growth deregulated cells is a tumor suppressive mechanism and, hence, confers a survival advantage. Even if transformed cells bypass senescence and reach crisis, the instability of these cells in the context of normal tissue and immune function in

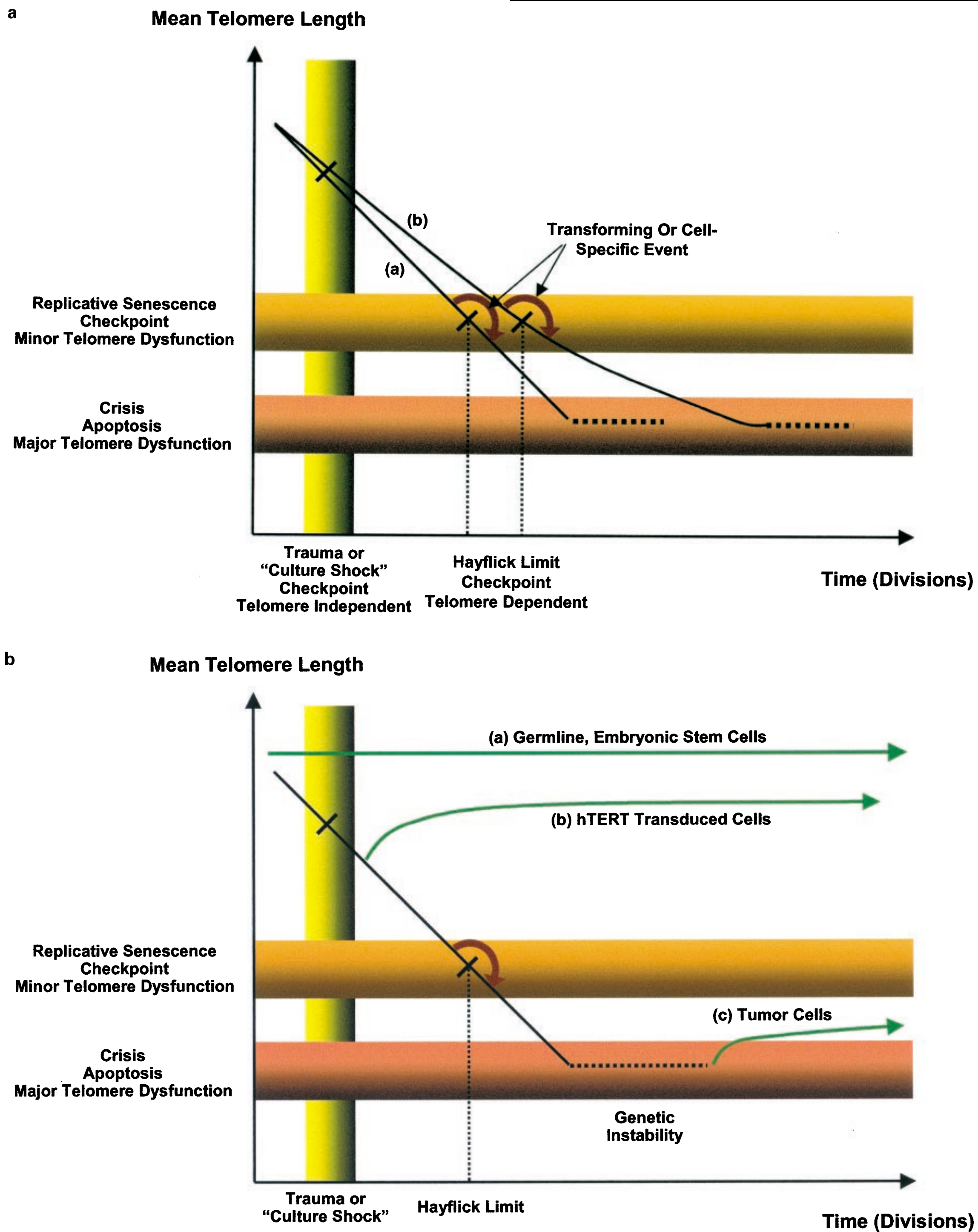

Figure 2 Schematic of the telomere hypothesis of cellular aging and immortalization. The relationship between telomere length and time, measured typically in cell divisions, is shown for mortal (**A**) and immortal (**B**) human cells. In normal telomerase-negative mortal cells from many tissues there exists at least two telomere-dependent mortality phases (horizontal bars). Replicative senescence is is characterized by a checkpoint arrest likely triggered by a DNA damage response due to critical telomere loss or uncapping on one or a few chromosome ends. If cells lack this checkpoint, or suffer a transforming growth control mutation, they can continue to divide, losing telomeric DNA until the crisis phase characterized by major telomere dysfunction, genetic instability, and apoptosis. Although many cells lack detectable telomerase and lose telomeric DNA in a linear fashion with cell divisions (line a), certain cells from highly proliferative tissues have low or transiently activated levels of telomerase. These cells have a slower rate of telomere loss and an extended, but still finite lifespan (line b). The vertical bar represents non-telomeric checkpoint arrest mechanisms seen with many human and murine cells placed in culture ('culture shock'), or when cells suffer non-lethal acute trauma or inappropriate growth conditions *in vitro* or *in vivo*. (**B**) illustrates telomere maintenance in: (a) immortal germline (reproductive) and embryonic stem cells, achieved by the normal constitutive expression of endogenous telomerase; (b) normal somatic cells immortalized by expression of ectopic (transduced) hTERT; and (c) tumor cells which have undergone growth control mutations and abnormal activation of endogenous telomerase. Based on Harley *et al.* (1990) and modified from Harley (1991)

young individuals more likely triggers their destruction than contributes to lethal tumor progression. This beneficial effect of telomerase repression early in life could offset a multitude of detrimental consequences late in life, beyond the normal period of reproductive fitness, when evolutionary selective pressures are greatly diminished. However, there is no quantitative or even theoretical analysis on this issue, to my knowledge.

The detrimental late-onset consequences of early telomerase repression fall into three categories. First, the telomere-dependent aging of normal cells, especially in areas of chronic stress, is expected to contribute to loss of function in all tissues containing or supported by proliferative cells. Second, aging cells may also contribute to an increased frequency of tumors through stimulating the proliferation of surrounding cells or through loss of tumor-restraining or tumor-killing functions. Finally, in old age the increasing fraction of senescent cells or transformed cells at crisis may also contribute to tumor initiation due to telomere dysfunction and genetic instability, as described in the telomerase knockout mouse, and suggested by the progression of myelodysplastic syndrome (Engelhardt *et al*., 2000) and the phenotype of dyskeratosis congenita.

Controlled telomerase activation therapies should not pose an unacceptable risk of cancer

The potential benefits of telomerase inhibition for cancer patients was described earlier and is reviewed elsewhere in this issue. However, the links between telomerase activation and tumor progression in animal models and humans raises the question of whether telomerase activation directed towards treatment of degenerative, age-related diseases, will pose an unacceptable risk of cancer. The answer of course depends upon many factors, including how telomerase is

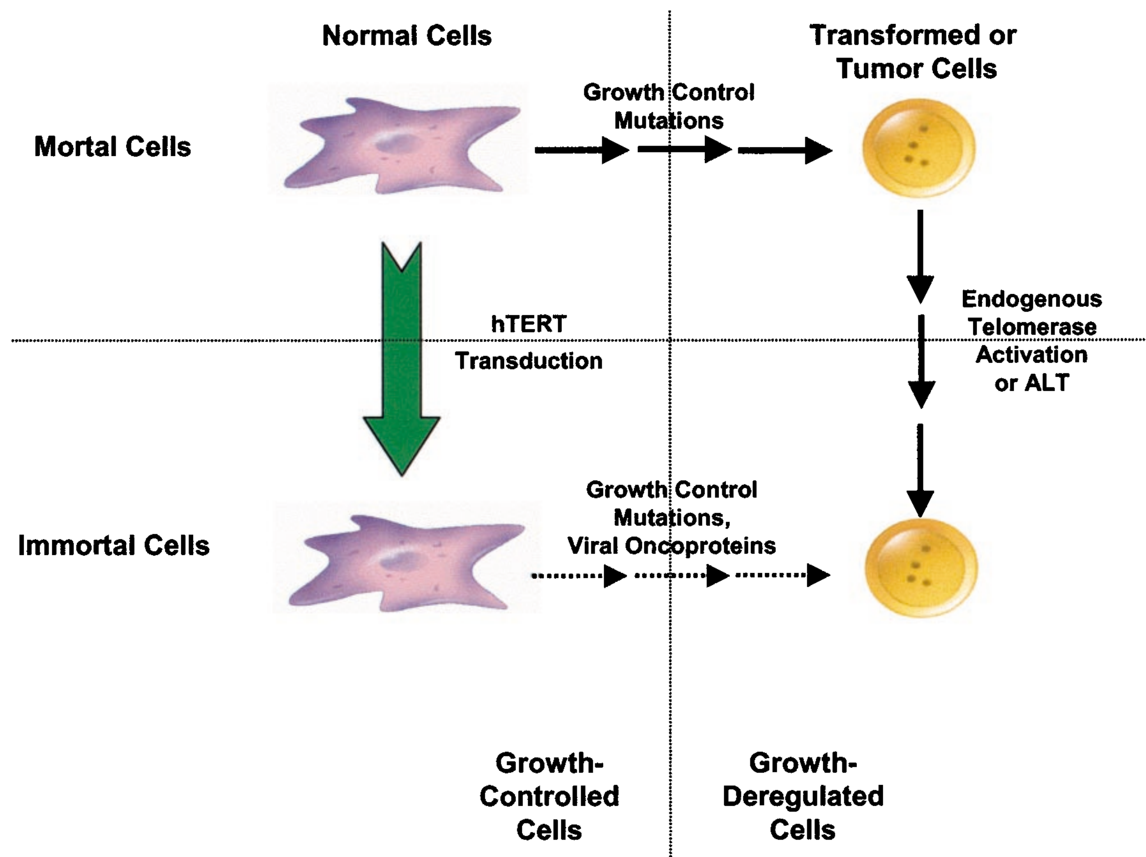

Figure 3 Diagram representing the orthogonal distinction between mortal and immortal cells on the one hand, and growth-controlled and growth-deregulated cells on the other. Growth-controlled normal mortal cells (top left) can be readily immortalized by hTERT transduction (large arrow), and remain growth-controlled (lower left). Immortalization of normal cells by spontaneous activation of endogenous hTERT is extremely rare, probably because of redundant repressive or regulatory pathways acting in concert. However, normal mortal cells may become growth deregulated through mutations, a process facilitated by aging and genetic instability triggered by telomere loss (top right). Such transformed cells may become immortal by activation of the endogenous hTERT gene, a process presumably also facilitated by genetic instability (bottom right). Transformed cells may also become immortal through the rare activation alternative (ALT) mechanisms of telomere maintenance. Spontaneous transition from hTERT transduced immortal normal cells to growth-deregulated transformed or tumorous cells has yet to be observed, but can be forced by expression of viral oncoproteins (broken arrows, bottom)

Table 1 hTERT immortalization of somatic cells

Cell type and donor age	*Tissue*	*Immortal*	*References*
Fibroblasts			
fetal	lung	yes	(McSharry *et al*., 2001)
fetal	lung	yes	(MacKenzie *et al*., 2000)
fetal	lung	yes[1]	(Franco *et al*., 2001)
newborn	foreskin	yes	(Bodnar *et al*., 1998)
newborn	foreskin	yes	(Vaziri and Benchimol, 1999)
adult			
normal	skin	yes	(Wyllie *et al*., 2000)
normal	?	yes	(Oullette *et al*., 2000)
normal	mammary	no[2]	(O'Hare *et al*., 2001)
Fanconi anemia	skin	yes[3]	(Oullette *et al*., 2000)
Blooms	skins	yes[3]	(Oullette *et al*., 2000)
Roberts	skin	yes[3]	(Oullette *et al*., 2000)
XP (E&V)	skin	yes[3]	(Oullette *et al*., 2000)
Werner	skin	yes[3]	(Oullette *et al*., 2000)
Werner	skin	yes[3]	(Choi *et al*., 2001)
Werner	skin	yes[3]	(Wyllie *et al*., 2000)
Progeria	skin	yes[3]	(Oullette *et al*., 2000)
Ataxia telangiectasia	skin	yes	(Wood *et al*., 2001)
Nijmegan breakage	skin	yes[3]	(Ranganathan *et al*., 2001)
Endothelial Cells			
newborn	umbilical vein	yes	(Yang *et al*., 1999)
adult	aortic artery	yes	(Yang *et al*., 1999)
adult	saphenous vein	yes	(Yang *et al*., 1999)
newborn	skin microvasc.	yes	(Yang *et al*., 1999)
adult	mammary	no[2]	(O'Hare *et al*., 2001)
adult	liver sinusoid	yes	(Salmon *et al*., 2000)
Keratinocytes			
newborn	foreskin	yes	(Ramirez *et al*., 2001)
newborn	foreskin	no[4]	(Farwell *et al*., 2000)
newborn	foreskin	no[4]	(Dickson *et al*., 2000)
newborn	foreskin	no[4]	(Kiyono *et al*., 1998)
newborn	foreskin	yes[5]	(Jiang, X.-R. unpublished data)
adult	skin	yes[5]	(Jiang, X.-R. unpublished data)
RPE cells, child	eye	yes	(Bodnar *et al*., 1998)
Other epithelial cells			
adult	bronchial	yes	(Chiu, C.-P. unpublished data)
adult	mammary	yes	(Ramirez *et al*., 2001)
adult	mammary	no[4]	(Farwell *et al*., 2000)
adult	mammary	no[4]	(Kiyono *et al*., 1998)
?	adenoid	no[4]	(Farwell *et al*., 2000)
?	throid	no[4,6]	(Jones *et al*., 2000)
Melanocytes, newborn	skin	yes[7]	(Bandyopadhyay *et al*., 2001)
Smooth muscle, adult	coronary artery	yes	(Chiu, C.-P, unpublished data)
Myoblasts/satellite cells			(Seigneurin-Venin *et al*., 2000a;
normal and DMD children	skeletal muscle	no[2]	Seigneurin-Venin *et al*., 2000b)
Lymphocytes	blood, CD8+	yes	(Hooijberg *et al*., 2000)
	blood, CD8+	no	(Migliaccio *et al*., 2000)
Adrenocortical cells			
bovine, transformed	adrenal gland	yes[8]	(Thomas *et al*., 2000)
Osteoblasts, adult	bone	yes	(Yudoh *et al*., 2001)
Islet cells (beta enriched)			
adult primary	pancreas	no[9]	(Halvorsen *et al*., 2000)
adult transformed	pancreas	yes[10]	(Halvorsen *et al*., 1999)

[1]26 clones examined, ≈1/3 immortalized; [2]hTERT + T-antigen required for immortalization; [3]Primary genetic mutant phenotype preserved; [4]cells grown on plastic; [5]two independant strains; [6]primary cells survived <3 doublings; [7]extended lifespan, not clear if immortal; [8]hTERT immortalized bovine T-antigen transformed cells and these cells had an apparently normal phenotype when transplanted into mice; [9]primary cells survived <15 doublings; [10]T-antigen transformed

activated, in what cells or tissues it is activated, the duration of the activation, the magnitude of the potential benefit, and the alternatives for the patient; in brief, the risk/benefit ratio. The most obvious way to minimize the potential risk of immortalizing a telomerase-negative tumor cell that might reside in the tissue targeted for telomerase therapy is to use transient or conditional telomerase activation. This would be possible with a pharmacological agent that stimulates telomerase in telomerase-competent cells, or with an hTERT gene therapy strategy that utilizes transient or regulated vector systems. The tumor risk could be further reduced by targeting tissues in which tumor progression is rare, and the risk/benefit ratio would be improved by targeting diseases for which effective, durable, alternative therapies are lacking. Targeting fibroblasts or keratinocytes in chronic skin ulcers, retinal pigmented epithelial cells in macular degeneration (Matsunaga *et al*., 1999), and certain immune cells in AIDS (Effros *et al*., 1996) has been considered.

The growing evidence that telomere-dependent replicative senescence may facilitate tumor initiation directly or indirectly, argues that telomerase activation may actually have a protective effect on tumor initiation or progression in elderly patients. Thus, telomerase activation could have a net beneficial effect when directed to cells in degenerative diseases, or even to immune cells in non-hematologic cancers. However, a cautious approach to telomerase activation therapy is still warranted until we more fully evaluate the risks through empirical data.

Conclusions

Telomerase is the critical enzyme in overcoming growth limitations due to telomere dysfunction, but it does not cause growth deregulation and hence is not an oncogene. There are, in fact, dozens of normal cell types that have been immortalized with telomerase without signs of cancerous changes, without altering differentiation capacity, and without altering pre-existing genetic abnormalities. These observations suggest a range of opportunities for the use of telomerase immortalized cells in research, disease modeling, and drug discovery, as well as telomerase activation for treatment of certain age-related diseases. Even though telomerase-mediated prevention of cell senescence and/or genetic instability of pre-crisis cells may reduce the initiation or progression of cancers, caution is still warranted in telomerase activation therapies, as the theoretical risk of immortalizing or extending the lifespan of existing tumor cells must be weighed against the potential benefits.

Killing tumor cells in cancer patients with telomerase-based strategies has never before looked so promising. Despite the complexities of telomere dynamics on cancer initiation and progression in mice, and unresolved questions in humans, it seems clear that 'remortalizing' a lethal tumor through effective and specific telomerase inhibition, especially for tumors with short telomeres, will improve a patient's prognosis. While advances continue to be made in the discovery of telomerase inhibitors, other approaches to specifically killing telomerase-positive tumor cells have achieved experimental support. hTERT promoter-driven suicide genes have proven effective *in vitro* and in animal models, and therapeutic hTERT vaccine strategies are now in human clinical studies.

Although further research in this exciting new area of biology will undoubtedly help clarify mechanisms of action and point to better, safer, and more cost-effective therapeutic approaches, it is gratifying to finally see the light at the end of the tunnel for the development of much-needed medicines based upon the telomere hypothesis of cell aging and immortalization.

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References

Amit M, Carpenter MK, Inokuma MS, Chiu C-P, Harris CP, Waknitz MA, Itskovitz-Eldor J and Thomson JA. (2000). *Dev. Biol.*, **227,** 271–278.
Bandyopadhyay D, Timchenko N, Suwa T, Hornsby PJ, Campisi J and Medrano EE. (2001). *Exp. Gerontol.*, **36,** 1265–1275.
Blackburn EH. (2001). *Cell*, **106,** 661–673.
Blackburn EH and Szostak JW. (1989). *Ann. Rev. Genet.*, **23,** 163–194.
Bodnar A, Kim NW, Effros RB and Chiu C-P. (1996). *Exp. Cell Res.*, **228,** 58–64.
Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu C-P, Morin GB, Harley CB, Shay JW, Lichtsteiner S and Wright WE. (1998). *Science*, **279,** 349–352.
Bryan TM, Marusic L, Bacchetti S, Namba M and Reddel RR. (1997). *Hum. Mol. Gen.*, **6,** 1–16.
Campisi J. (1997). *Eur. J. Cancer*, **33,** 703–709.
Chen J-L, Blasco MA and Greider CW. (2000). *Cell*, **100,** 503–514.
Choi D, Whittier PS, Oshima J and Funk WD. (2001). *FASEB J.*, **15,** 1014–1020.
Cong YS and Bachetti S. (2000). *J. Biol. Chem.*, **275,** 35665–35668.
Dessain SK, Yu H, Reddel RR, Beijersbergen RL and Weinberg RA. (2000). *Cancer Res.*, **60,** 537–541.
Dickson MA, Hahn WC, Ino Y, Ronfard V, Wu JY, Weinberg RA, Louis DN, Li FP and Rheinwald JG. (2000). *Mol. Cell. Biol.*, **20,** 1436–1447.
Effros RB, Allsopp R, Chiu C-P, Hausner MA, Hirji K, Wang L, Harley CB, Villeponteau B, West MD and Giorgi JV. (1996). *AIDS*, **10,** F17–F22.

Engelhardt M, Mackenzie K, Drullinsky P, Silver RT and Moore MA. (2000). *Cancer Res.,* **60,** 610–617.

Farwell DG, Shera KA, Koop JI, Bonnet GA, Matthews CP, Reuther GW, Coltrera MD, McDougall JK and Klingelhutz AJ. (2000). *Am. J. Pathol.,* **156,** 1537–1547.

Feng J, Funk WD, Wang S-S, Weinrich SL, Avilion AA, Chiu C-P, Adams RR, Chang E, Yu J, Le S, West MD, Harley CB, Andrews WH, Greider CW and Villeponteau B. (1995). *Science,* **269,** 1236–1241.

Franco S, MacKenzie KL, Dias S, Alvarez S, Rafii S and Moore MA. (2001). *Exp. Cell Res.,* **268,** 14–25.

Funk WD, Wang CK, Shelton DN, Harley CB, Pagon GD and Hoeffler WK. (2000). *Exp. Cell. Res.,* **258,** 270–278.

Gonzalez-Suarez E, Samper E, Flores JM and Blasco MA. (2000). *Nat. Genet.,* **26,** 114–117.

Goytisolo FA, Samper E, Martin-Caballero J, Finnon P, Herrera E, Flores JM, Bouffler SD and Blasco MA. (2000). *J. Exp. Med.,* **192,** 1625–1636.

Greider CW. (1996). *Annu. Rev. Biochem.,* **65,** 337–365.

Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H and de Lange T. (1999). *Cell,* **97,** 503–514.

Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JHM, Meyerson M and Weinberg RA. (1999). *Nat. Med.,* **5,** 1164–1170.

Halvorsen RL, Leibowitz G and Levine F. (1999). *Mol. Cell. Biol.,* **19,** 1864–1870.

Halvorsen TL, Beattie GM, Lopez AD, Hayek A and Levine F. (2000). *J. Endrocrinol.,* **166,** 103–109.

Harley CB. (1991). *Mut. Res.,* **256,** 271–282.

Harley CB, Futcher AB and Greider CW. (1990). *Nature,* **345,** 458–460.

Harley CB, Kim NW, Prowse KR, Weinrich SL, Hirsch KS, West MD, Bacchetti S, Hirte HW, Counter CM, Greider CW, Wright WE and Shay JW. (1994). *Cold Spring Harbor Symp. Quant. Biol.,* **59,** 307–315.

Hemann M, Strong M, Hao L and Greider C. (2001). *Cell,* **107,** 67–77.

Herrera E, Samper E, Martin-Caballero J, Flores JM, Lee H-W and Blasco MA. (1999). *EMBO J.,* **18,** 2950–2960.

Hiyama E, Hiyama K, Yokoyama T, Mitsuura Y, Piatyszek MA and Shay JW. (1995a). *Nature Med.,* **1,** 249–255.

Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyamas E, Piatyszek MA, Shay JW, Ishioka S and Yamakido M. (1995b). *J. Immunol.,* **155,** 3711–3715.

Hooijberg E, Ruizendaal JJ, Snijders PJF, Kueter EWM, Walboomers JMM and Spits H. (2000). *J. Immunol.,* **165,** 4239–4245.

Jiang X-R, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, Beeche M, Bodnar AG, Wahl GM, Tlsty TD and Chiu C-P. (1999). *Nat. Genet.,* **21,** 111–114.

Jones CJ, Kipling D, Morris M, Hepburn P, Skinner J, Bounacer A, Wyllie FS, Ivan M, Bartek J, Wynford-Thomas D and Bond JA. (2000). *Mol. Cell. Biol.,* **20,** 5690–5699.

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL and Shay JW. (1994). *Science,* **266,** 2011–2014.

Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA and Klingelhutz AJ. (1998). *Nature,* **396,** 84–88.

Komata T, Kondo Y, Kanzawa T, Hirohata S, Koga S, Sumiyohi H, Srinivasula S, Barna B, Germano I, Takakura M, Inoue M, Alnemri E, Shay JW, Kyo S and Kondo S. (2001). *Cancer Res.,* **61,** 5796–5802.

Krtolica A, Parrinello S, Lockett S, Desprez PY and Campisi J. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 12072–12077.

Lee H-W, Blasco MA, Gottlieb G, Horner II JW, Greider CW and DePinho RA. (1998). *Nature,* **392,** 569–574.

MacKenzie KL, Franco S, May C, Sadelain M and Moore MAS. (2000). *Exp. Cell. Res.,* **259,** 336–350.

Majumdar AS, Hughes DE, Lichtsteiner SP, Wang Z, Lebkowski JS and Vasserot AP. (2001). *Gene Ther.,* **8,** 568–578.

Matsunaga H, Handa JT, Aotaki-Keen A, Sherwood SW, West MD and Hjelmeland LM. (1999). *Invest. Ophtalmol. Vis. Sci.,* **40,** 197–202.

McSharry BP, Jones CJ, Skinner JW, Kipling D and Wilkinson GWG. (2001). *J. Gen. Virol*.**,** **82,** 855–863.

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.

Migliaccio M, Amacker M, Just T, Reichenbach P, Valmori D, Cerottini J-C, Romero P and Nabholz M. (2000). *J. Immunol.,* **165,** 4978–4984.

Minev B, Hipp J, Firat H, Schmidt H, Langlade-Demoyen P and Zanetti M. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 4796–4801.

Mitchell JR and Collins K. (2000). *Mol. Cell.,* **6,** 361–371.

Nair, S.K., Hiser, A., Boczkowski, D., Majumdar, A., Naoe M, Lebkowski J, Vieweg J and Gilboa E. (2000). *Nat. Med.,* **6,** 1011–1017.

Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB and Cech TR. (1997). *Science,* **277,** 955–959.

O'Hare MJ, Bond J, Clarke C, Takeuchi Y, Atherton AJ, Berry C, Moody J, Silver ARJ, Davies DC, Alsop AE, Neville AM and Jat PS. (2001). *PNAS,* **98,** 646–651.

Ouellette MM, McDaniel LD, Wright WE, Shay JW and Schultz RA. (2000). *Hum. Mol. Gen.,* **9,** 403–411.

Poole JC, Andrews LG and Tollefsbol TO. (2001). *Gene,* **269,** 1–12.

Ramirez RD, Morales CP, Herbert B-S, Rohde J, Passons C, Shay JW and Wright WE. (2001). *Genes Dev.,* **15,** 398–403.

Ranganathan V, Heine WF, Ciccone DN, Rudolph KL, Wu X, Chang S, Hai H, Ahearn IM, Livingston DM, Resnick I, Rosen F, Seemanova E, Jarolim P, DePinho RA and Weaver DT. (2001). *Curr. Biol.,* **11,** 962–966.

Reynolds CP, Zuo JJ, Kim NW, Wang H, Lukens J, Matthay KK and Seeger RC. (1997). *Eur. J. Cancer,* **33,** 1929–1931.

Rudolph KL, Chang S, Lee H-W, Blasco M, Goettlieb GJ, Greider C and DePinho RA. (1999a). *Cell,* **96,** 701–712.

Rudolph KL, Chang S, Millard M, Schreiber-Agus N and DePinho RA. (1999b) *Science,* **287,** 1253–1258.

Rudolph KL, Millard M, Bosenberg MW and DePinho RA. (2001). *Nat. Genet.,* **28,** 155–159.

Salmon P, Oberholzer J, Occhiodoro T, Morel P, Lou J and Trono D. (2000). *Mol. Ther.,* **2,** 404–414.

Seigneurin-Venin S, Bernard V, Ouellette MM, Mouly V, Wright WE and Tremblay J. (2000a). *Biochem. Biophys. Res. Commun.,* **7,** 362–369.

Seigneurin-Venin S, Bernard V and Tremblay JP. (2000b). *Gene Thera.,* **7,** 619–623.

Smith S and de Lange T. (2000). *Curr. Biol.,* **10,** 1299–1302.

Smogorzewska A, van Steensel B, Bianchi A, Oelmann S, Schaefer MR, Schnapp G and de Lange T. (2000). *Mol. Cell. Biol.,* **20,** 1659–1668.

Taylor RS, Ramirez RD, Ogoshi M, Chaffins M, Piatyszek MA and Shay JW. (1996). *J. Invest. Dermatol.,* **106,** 759–765.

Thomas M, Yang L and Hornsby PJ. (2000). *Nat. Biotech.,* **18,** 39–42.

Ulaner GA and Giudice LC. (1997). *Mol. Hum. Reprod.,* **3,** 769 – 773.

Ulaner GA, Hu J-F, Hu TH, Giudice LC and Hoffman AR. (1998). *Cancer Res.,* **58,** 4168 – 4172.

Usselmann B, Newbold M, Morris AG and Nwokolo CU. (2001). *Am. J. Gastroenterol.,* **96,** 1106 – 1112.

Vaziri H and Benchimol S. (1999). *Oncogene,* **18,** 7676 – 7680.

von Zglinicki T. (2000). *Ann. NY Acad. Sci.*, **??,** 99 – 110.

Vonderheide RH, Hahn WC, Schultze JL and Nadler LM. (1999). *Immunity,* **10,** 673 – 679.

Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ and Dokal I. (2001). *Nature,* **413,** 432 – 435.

Weng N-P, Granger L and Hodes RJ. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 10827 – 10832.

Weng N-P, Levine BL, June CH and Hodes RJ. (1996). *J. Exp. Med.,* **183,** 2471 – 2479.

Wong K-K, Chang S, Weiler SR, Ganesan S, Chaudhuri J, Zhu C, Artandi SE, Rudolph KL, Gottlieb GJ, Chin L, Alt FW and DePinho RA. (2000). *Nat. Gen.,* **26,** 85 – 88.

Wood LD, Halvorsen TL, Dhar S, Baur JA, Pandita RK, Wright WE, Hande MP, Calaf G, Hei TK, Levine F, Shay JW, Wang JJ and Pandita TK. (2001). *Oncogene,* **20,** 278 – 288.

Wright WE, Piatyszek MA, Rainey WE, Byrd W and Shay JW. (1996). *Dev. Genet.,* **18,** 173 – 179.

Wright WE and Shay JW. (2001). *Curr. Opin. Genet. Dev.,* **11,** 98 – 103.

Wyllie FS, Jones CJ, Skinner JW, Haughton MF, Wallis C, Wynford-Thomas D, Faragher RGA and Kipling D. (2000). *Nat. Genet.,* **24,** 16 – 17.

Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, Bronstein A, Chiu C-P and Herron GS. (1999). *J. Biol. Chem.,* **274,** 26141 – 26148.

Yudoh K, Matsuno H, Nakazawa F, Katayama R and Kimura T. (2001). *J. Bone Miner. Res.,* **16,** 1453 – 1464.

Zhang X, Mar V, Harrington L and Robinson MO. (1999). *Genes Dev.,* **13,** 2388 – 2399.