

Review Article

Telomere and telomerase in hematological disorders Focusing on bone marrow failure syndromes and hematological malignancies

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ABSTRACT

We review the present knowledge of telomeres and telomerase with special attention to their role in hematological disorders especially bone marrow failure syndromes including acquired aplastic anemia and myelodysplastic syndromes, as well as acute and chronic myeloid leukemia. The current understanding on the role of telomere and telomeres dysfunctions in hematological disorders leads us to a better understanding on the pathology of the diseases as well as considering some possibilities to employ the measurement of telomere length and telomere activity in disease prognostication. Several treatment options targeting telomere and telomerase being developed are also reviewed.

Keywords: *telomere-telomerase-bone marrow failure syndromes-hematological malignancies*

Telomeres

Telomeres are the non-encoding regions of DNA capping the ends of chromosomes, in association with various proteins, known as shelterin. The DNA that forms the telomere consists of thesequence (5'-TTAGGG-3')_n, which is referred to as a “telomeric repeat” due to its tandem repeat over 5 to 15 kilobases (kb). A single-stranded 3'-hydroxyl overhang is generated by the catalytic addition of telomeric repeats to the 3' end and by post replicative processing of the lagging strand.¹ This single stranded overhang folds back and invades the double-stranded telomeric helix, forming the T loop in order to avoid being recognized as a double-strand break and corrected by DNA repair machineries.² Thus, telomeres function

to guard chromosomes against degradation, fusion, and rearrangements during DNA replication.³ When telomeres become very short, they signal the arrest of cell proliferation, senescence, and apoptosis. The cells whose telomeres shorten to a “critical length” enter a stage termed replicative senescence whereby cell division is prevented.¹

The shelterin complex is formed by the double-stranded DNA binding proteins. These proteins include TRF1, TRF2, TIN2, POT1, TPP1, and RAP1. TRF1 is thought to regulate telomere length by preventing the elongation of telomeres once they reach a critical size. TRF2 appears to be important for stabilizing the chromosome ends by associating with the 3' overhang and suppressing end-to-end fusions between chromosomes. Other

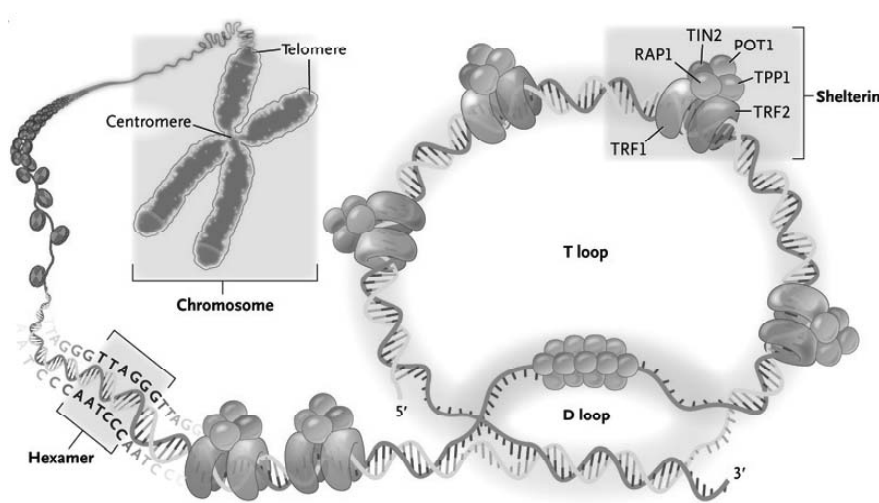


Figure 1. Telomeres are located at the ends of linear chromosomes; they are composed of hundreds to thousands of tandem DNA repeat sequences: hexameric TTAGGG in the leading strand and CCCTAA in the lagging strand in humans. Protective proteins associated with telomere DNA are collectively termed shelterin (TRF1, TRF2, TIN2, POT1, TPP1, and RAP1). The 3' end of the telomeric leading strand terminates as a single-stranded overhang, which folds back and invades the double-stranded telomeric helix, forming the T loop.¹

additional proteins can bind indirectly to telomeres, often via TRF1 and TRF2, and together these proteins function to regulate telomere homeostasis. They consist of RAP1-a binding partner of TRF2, POT1- a single-stranded DNA binding protein; and the two bridging proteins, TIN2 and TPP1.⁴ Not only do these proteins function in protecting the chromosome end, they also function in telomere length regulation. Any mutations reducing their expression or impairing their binding to DNA result in telomere erosion. Telomere length is maintained within a strict range throughout cell division, suggesting a negative feedback loop involving the shelterin complex. Due to the exquisite specificity of these DNA binding proteins, the amount of shelterin protein bound to telomeres is roughly proportional to their length.²

Telomerase

Telomerase is a DNA polymerase required to catalyze DNA synthesis to maintain telomere length. It is composed of two essential subunits, human telomerase RNA component (hTERC) and human telomerase catalytic component (hTERT). hTERT uses the telomerase RNA component (TERC) as a template to synthesize telomere DNA.¹ The assembly of a functional telomerase holoenzyme complex also requires other telomere- and/or telomerase-associated proteins (e.g., dyskerin, NOP10, GAR1, NHP2) to stabilize the complex.⁵ The structural organization of hTERT can be divided into three functional domains which at the N terminus are the telomerase-specific domains required for functional assembly of the enzyme complex by mediating TERT interaction with its TER RNA partner and the

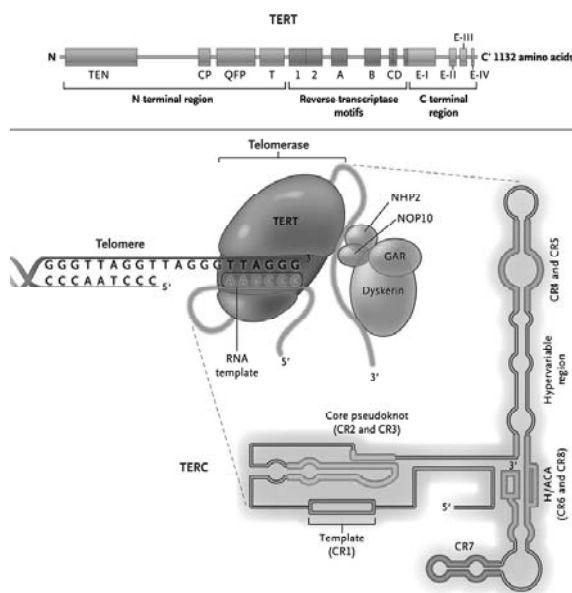


Figure 2. The Telomerase Complex and Its Components. The enzyme telomerase reverse transcriptase (TERT), its RNA component (TERC), the protein dyskerin, and other associated proteins (NHP2, NOP10, and GAR1) are shown.¹

homodimerization of the protein (i.e., TERT protein-protein interaction). The functional reverse transcriptase (RT) domain is almost centrally located. The C-terminal domains of TERTs are also required for telomerase-specific enzymatic activity and/or in the telomeric nucleotide addition processivity process.⁵

Telomerase is regulated by a wide variety of genes or multifactorial. Removal or reduced regulation of the hTERC subunit leads to a loss of telomerase activity, erosion of telomeres and inhibition of cellular growth. Mutation of the hTERC gene has been described in distinct autosomal dominant disorder dyskeratosis congenita.⁶ Besides its function as a binding site for the SP1 transcription factor, the hTERT promoter also provides other binding sites for various transcription

factors and hormone responsible elements. hTERT is repressed by retinoblastoma protein (Rb) and cyclin-dependent kinase inhibitor p21WAF1. Conversely, c-Myc activates TERT gene expression. hTERT phosphorylation is another mechanism of telomerase activity regulation. It is expressed mainly in embryonic and adult stem cells, highly proliferative cells such as mature lymphocytes, and in cancer cells, but not in most mature cells.⁵ While hTERC is expressed in relatively identical amount in embryonic and somatic tissues, the expression of hTERT is precisely regulated and undetectable in many somatic cells. This means that the expression of hTERT is the limiting step in telomerase activation.⁶

Quantitative assays are available for telomere length and telomerase enzymatic activity. Telomeres can be visualized by fluorescent in situ hybridization (FISH) of individual cells and in-flow cytometry of specific cell populations (Young ASH). The length of telomere can be determined by a modification of Southern blotting in which the analysis of chromosome terminal restriction fragments (TRFs), as visualized with a radiolabeled telomeric repeat probe, provides the average lengths of all telomeres in a cell population. It also can be measured by quantitative polymerase chain reaction amplification assays. Telomerase activity can be measured in vitro by a sensitive and efficient polymerase chain reaction (PCR)-based detection method, also known as telomeric repeat amplification protocol (TRAP).⁷

Telomere and on cogenesis

Most cancers show gross derangement in chromosome numbers. Telomere attrition has also been proposed as a mechanism for the

loss or gain of chromosomes. When telomere maintenance is disrupted in yeast, the few cells that escape senescence show chromosome abnormalities, end-to-end fusions, and consequent formation of dysentric and circularized chromosomes. In the absence of telomerase, genetic lesions increase due to terminal chromosome deletions and repeated cycles of break–fusion–bridge rearrangements. In “knock-out” mice that lack the RNA template component of telomerase, telomeres shorten progressively with each generation, producing chromosomal instability by end-to-end fusions.^{9,10} Most unstable cells are removed by apoptosis, but they can be rescued if DNA damage is not inadequately monitored: in mTERC^{-/-} mice that also are deficient in the tumor suppressor gene p53, a variety of carcinomas appear associated with nonreciprocal translocations, as seen in human cancers.¹¹

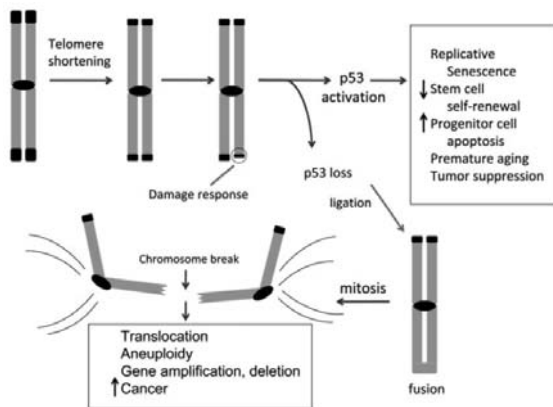


Figure 3. Telomere shortening activates p53 and drives formation of epithelial cancers through gene amplification and deletion. Telomeres shorten progressively with cell division due to the end-replication problem in settings of insufficient telomerase, including in human fibroblasts, aging tissues, early cancers and diseases of high cellular turnover. Critical telomere

shortening compromises the telomere cap and results in a DNA damage response that activates the p53 tumor suppressor protein. This activation of p53 induces replicative senescence in cultured human fibroblasts, impairs stem cell self-renewal, induces apoptosis in tissue progenitor cells, causes premature aging and strongly suppresses tumor formation. If p53 is mutated or deleted, these responses to telomere dysfunction are mitigated and chromosomal fusions are tolerated. The generation of fused chromosomes results in dysentric chromosomes (chromosomes with two centromeres) and when these attach to opposite spindle poles, chromosome breakage occurs. These broken ends serve as potent catalysts for translocations, focal amplifications and focal deletions. Such CNAs drive development of carcinomas and explain the widespread gene copy number changes seen in human cancers.¹⁵

In humans, telomere length has been linked to malignant transformation—to the onset of cancer—in several diseases. When telomeres were first noted to be short in colorectal cancer, telomere loss was speculated to contribute to tumor genesis and genetic instability.¹² Telomerase deficiency has been reported in the histologically normal mucosa of inflammatory bowel disease. Losses of chromosomes in no dysplastic tissue of ulcerative colitis patients was correlated with telomere shortening and associated with the appearance of anaphase bridges, especially in patients who progressed to cancer.¹³ The major risk factor for esophageal cancer is the chronic inflammation of Barrett’s esophagus, the result of years of exposure to gastroesophageal reflux. Leukocyte telomere length at first

presentation is inversely proportional to the risk of later esophageal cancer, hypothesized to reflect a genetic predisposition to repair with persistent oxidative stress.¹⁴ Telomerase has also been reported to be up regulated in more than 90% of invasive breast cancers.¹⁵ An epidemiology study namely the Long Island Breast Cancer Study Project conducted among 1,067 cases and 1,110 controls, provided the strongest evidence to date that breast cancer risk may be affected by telomere length among premenopausal women or women with low dietary intake of antioxidants or antioxidant supplements.¹⁶ An analysis of the relative telomere length (RTL) of peripheral blood cells in relation to breast cancer incidence and prognosis including 265 newly diagnosed breast cancer patients and 446 female controls resulted that long RTL was a significant independent negative prognostic factor (hazards ratio, 2.92; 95% CI, 1.33–6.39; P = 0.007) in breast cancer patients with advanced disease.¹⁶

Telomere and telomerase in hematological disorders

Telomere and telomerase in bone marrow failure syndromes

Dyskeratosis congenita is a rare constitutional bone marrow failure syndrome stereotypically characterized by mucocutaneous abnormalities (nail dystrophy, hyperpigmentation, and leukoplakia) and aplastic anemia in childhood. Patients with dyskeratosis are at increased risk for malignancies, pulmonary fibrosis, and liver cirrhosis.¹⁷ The genetic basis study of dyskeratosis congenita found X-linked dyskeratosis congenita to associate with mutations in the DKC1 gene

which produces dyskerin associated with TERC. Patients' leukocytes showed reduced telomerase activity which explains the erosion of their chromosomes. The discovery that autosomal dominant dyskeratosis is caused by heterozygous mutations or large deletions in TERC established dyskeratosis congenita as a disease of telomerase insufficiency.¹⁸ Homozygous mutations in TERT43 and in NOP10, a telomerase-associated protein, were described in some families with autosomal recessive dyskeratosis congenita.¹⁹ More recently, mutations in TIN2, which encodes TIN2, a shelterin protein that caps and protects the telomeres, have been identified in a third of patients with dyskeratosis congenita, further implicating abnormal telomere maintenance in the pathophysiology of the disease.²⁰

In acquired aplastic anemia, an immune pathophysiology that targets hematopoietic stem cells has been inferred in most cases of acquired aplastic anemia from the hematologic response to immunosuppressive therapies; failure to respond to such treatments might be due to a non-immune etiology or to properties of the hematopoietic compartment, a quantitatively severe loss of stem cell number or a qualitative abnormality of stem and progenitor cells.²¹ When some patients with acquired aplastic anemia were found to have accelerated leukocyte telomere attrition, the cause was initially presumed to be secondary, a physiologic response to stem cell "stress."²²⁻²⁴ The discovery of the genetic basis of telomere erosion in dyskeratosis congenita made it plausible that some patients with acquired marrow failure also might have abnormal telomerase function as a basis for telomere shortening. The functional consequences of the genetic changes in TERC were reduced telomerase activity of patient primary cells, very short telomeres of leukocytes, and

low telomerase activity in vitro.²³ TERC mutations in either dyskeratosis congenita or acquired aplastic anemia are similar at the molecular level and produce the same effects on telomerase function, despite the diversity of clinical phenotypes (Table 1); an exception is large TERC gene deletions observed in dyskeratosis congenita families only.¹⁸ In cohort studies, the frequency of TERC mutations was low (approximately 4% of all patients with acquired aplastic anemia).^{26,27} Careful study of some families showed that healthy relatives of patients carrying TERC mutations also had short telomeres, some mild hematologic abnormalities (macrocytosis, mild anemia, thrombocytopenia, or granulocytopenia), reduced progenitor cells in peripheral blood, increased serum hematopoietic growth factors, and hypoplastic bone marrows.²⁶ One report that possibly explained potential clinical relevance of sometimes modest hematologic findings in healthy relatives of patients carrying TERC mutations was dramatically reported in hematopoietic stem cell graft failure in one proband, whose unknowingly affected sibling donor provided only very low numbers of CD34 cells from both marrow and mobilized blood collected in multiple leukaphereses.²⁸ A study on mutations in TERT, the gene encoding the telomerase reverse transcriptase itself, found that approximately 4% of patients with apparently acquired aplastic anemia had TERT mutations that disrupted telomerase activity, causing short telomeres of leukocytes and a hematopoietic stem cell compartment of limited proliferative capacity.²⁹ Several patients with TERT mutations also have a family history of blood dyscrasias, especially myelodysplastic syndrome evolving to acute myeloid leukemia, further suggesting a common genetic background for these disorders. As with TERC, apparently healthy relatives

with TERT mutations had short telomeres and reduced hematopoietic function.²⁹ The association between TERT mutations and aplastic anemia has been confirmed.^{29,30} A few patients with marrow failure have genetic variants and specific haplotypes for genes coding for shelterin components (TERF1 and TERF2) that might contribute to disease by disrupting the telomere homeostasis.³¹

Despite the various mutations reported in the telomerase complex, the question whether mutations sufficient to cause disease remains due to great variability is in the phenotype. Various clinical outcomes related with mutations in telomerase complex genes including isolated aplastic anemia, isolated pulmonary fibrosis or hepatic cirrhosis or multiorgan dyskeratosis congenita. The same mutation either in TERC or TERT in a single pedigree can associate with aplastic anemia in one patient, and pulmonary fibrosis or hepatic cirrhosis in another, suggesting that other factors contribute to organ damage. As an example, telomerase-mutant patients with pulmonary fibrosis often have a smoking history, suggesting this environmental insult as a trigger. Conversely, many relatives of patients with aplastic anemia with the same telomerase mutation have low telomerase activity, short telomeres, a hypoplastic bone marrow, and reduced hematopoietic function, but they are clinically healthy and asymptomatic.^{28,29} Although disease anticipation may play a role in determining manifestation, the hypoplastic bone marrow of the TERC- or TERT-mutant individuals appears capable of maintaining hematopoiesis under normal conditions, but may be hypothesized to be more susceptible to environmental injury, such as viral infections, drug exposure, or immune attack. Low telomerase function may result in a quantitative deficiency by reducing the

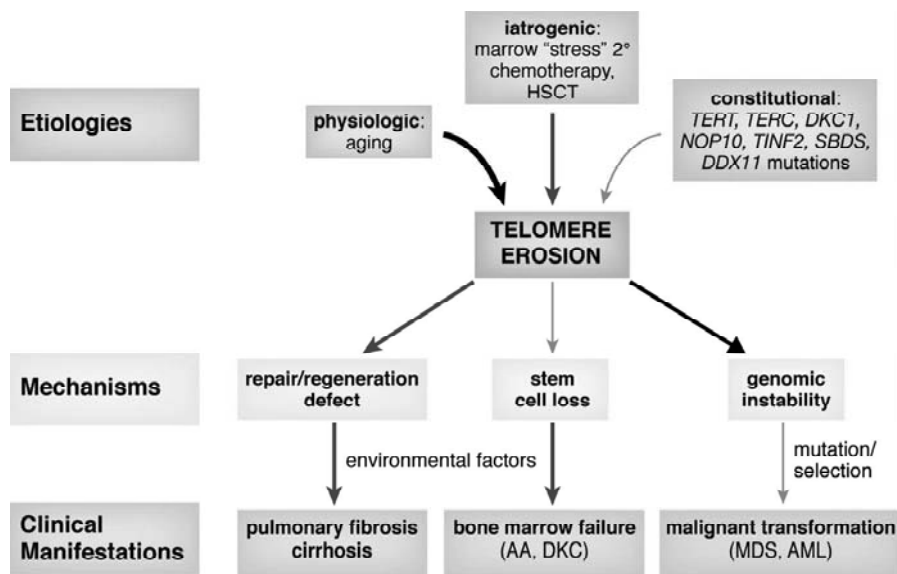


Figure 4. Proposed model for the role of dysfunctional and short telomeres in the pathogenesis of human disease. Aging, bone marrow stress, and genetic factors, such as mutations in telomerase complex genes (*TERT*, *TERC*, *DKC1*, and *NOP10*), shelterin components (*TINF2*, *TERF1*, and *TERF2*), or in other genes (*SBDS* and *DDX11*) produce progressive telomere erosion. Excessive telomere shortening results in defective cell proliferation, senescence, apoptosis, and genomic instability. Environmental factors, such as viruses, drugs, smoking, or asbestos exposure, may contribute to telomere shortening as well as injury to an organ with limited regeneration capacity, thus triggering disease development (aplastic anemia, pulmonary fibrosis, and hepatic cirrhosis). Short telomeres also promote genomic instability, breakage-fusion-bridge cycles, and aneuploidy, which can lead to myelodysplasia (MDS) or leukemia (AML).¹

number of hematopoietic stem cells able to maintain hematopoiesis, as well as a qualitative defect by impairing hematopoietic stem cell regeneration. Higher serum interferon γ and a limited T-cell receptor (TCR) $V\beta$ usage in telomerase mutant patients—similar to the skewed T-cell population typically observed in patients with immune acquired aplastic anemia³²—consistent with oligoclonal T-cell expansion and immune destruction of marrow as pathophysiologic had also been reported in these patients also.³¹ A telomerase mutation does not appear to be sufficient to determine aplastic anemia, but healthy relatives in which telomeres are short and/or a mutation need to

be further observed in long-term prospective studies.²⁰ Since the number of acquired aplastic anemia patients with identified mutation of either *TERC* or *TERT* genes or related genes are much fewer than those with short telomeres, it is suggested that other genetic lesions or environmental factors also may contribute to accelerated telomere erosion. In addition to aging, some environmental factors are known to cause telomere attrition. Smoking³⁴ and even psychological stress³⁵ have correlated with leukocyte telomere shortening. Over demanded or “stressed” hematopoiesis also can result in telomere erosion. Excessive

telomere shortening has been observed in the first years after allogeneic bone marrow transplantation in comparison with donor leukocytes, consistent with increased stem and progenitor turnover to replenish the bonemarrow.³⁶ Chemotherapy for solid tumors also causes myelotoxicity, requiring increased hematopoietic regeneration to recover blood cell counts, and premature telomere shortening has been observed following multiple cycles of cytotoxic drug therapy.³⁷ These observations are consistent with a contribution of “stress” hematopoiesis secondary to environmental factors to telomere shortening in aplastic anemia. However, the degree of telomere shortening produced in these clinical circumstances has been mild, less than 1 kb erosion in telomere lengths, as compared with the extreme attrition observed in individual mutations in telomerase deficiency (usually more than 3 kb). Other bone marrow failure syndromes involving telomeres shortening including Fanconi anemia, werner syndrome, Bloom

syndrome, Nijmegen breakage syndrome, and Shwachman-Diamond syndrome.²⁰

Telomere and telomerase in hematologic neoplasia

Normal hematopoietic cells express telomerase activity, however the presence of telomerase does not necessarily imply stable and thus unchanging telomere length. Therefore, before considering the telomere and telomerase status of leukemias and lymphomas we need to understand telomerase activity in normal hematopoietic cells as seen in Figure 5. The level of telomerase activity was different in human HSCs and their differentiate progeny. In contrast to somatic cells that lack telomerase activity, it is clear that many hematopoietic cells are telomerase-competent. Primitive HSCs are likely to be quiescent most of the time and thus the population of cells exhibits a low level of telomerase activity. However, upon stimulation to proliferate, telomerase activity

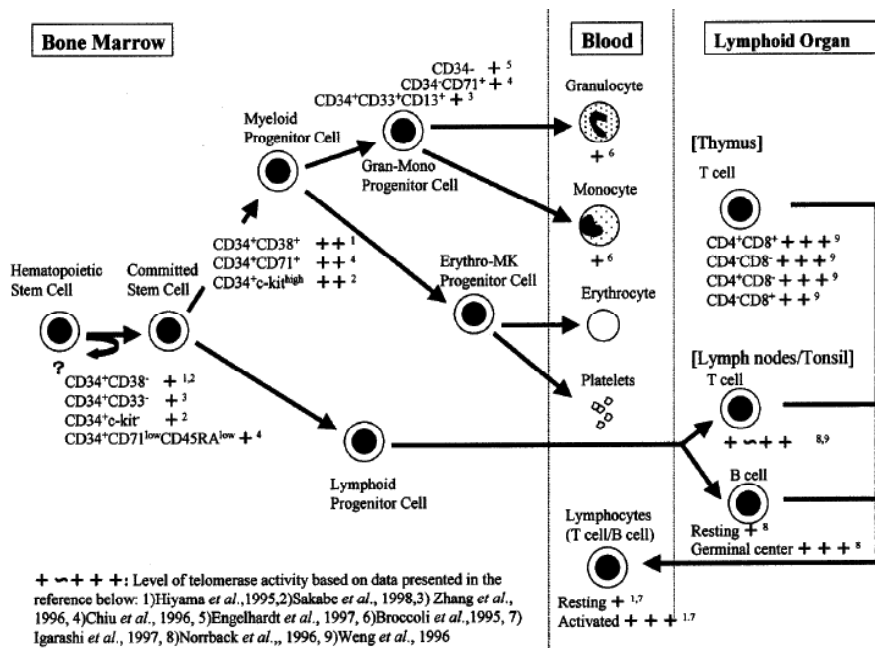


Figure 5. Telomerase activity in normal hematopoietic cell populations³⁸

appears to be upregulated in their immediate progeny and may help to slow down the rate of telomere erosion. The more mature cells then become quiescent again and down-regulate telomerase activity. The telomeres of HSCs shorten, probably due to inadequate levels of telomerase activity that slow but do not prevent telomere erosion. Both T cells and monocyte/B cells in peripheral blood also had low telomerase activity which was elevated when either cell type was cultured with mitogen. The highest in vivo telomerase expression in normal T cells is present in thymus, followed by T cells in the tonsil. The germinal center B-cells also show high telomerase activity. Thus, telomerase may play a permissive role in T cell and B cell development and in determining the capacity of lymphoid cells for cell division and clonal expansion. In summary, HSCs and lymphocytes are telomerase competent and mortal. The gradual telomere loss with aging and rapid cycling of HSCs or lymphocytes might contribute to immunosenescence, exhausted hematopoiesis, and increased likelihood of malignant transformation. Another interesting clinical aspect of these findings is the future possibility of selectively upregulated telomerase and controlling telomere length in certain cell types in order to achieve a delayed induction of replicative senescence. (reviewed in ref 38).

Acute Myeloid Leukemia and Myelodysplasia Syndrome

The overall importance of telomerase in the pathogenesis of AML has recently been confirmed by the demonstration that hTERT is necessary for growth of primary AML cells in a mouse model.³⁷ A number of studies have investigated telomerase activity and telomere

length in mononuclear cells (MNC) from patients with MDS and AML (reviewed in ref.39). Calado *et al* examined three cohorts of AML patients who show no physical signs of DC for sequence variation in the hTERT and hTERC genes. They identified three novel missense mutations in hTERT, and, while the V299M sequence change did not seem to affect telomerase enzymatic activity when tested by the TRAP assay, both the P65A and R522K mutations conferred dramatic defects. Surprisingly, they also identified three AML patients who are homozygous for sequence changes previously identified in a heterozygous state in AA patients and controls (A1062T and del441E). Thus, it appears that hTERT gene variants have low penetrance and are carried in patients with a wide variety of disorders. This phenomenon can be explained if short telomeres, as opposed to mutation status of telomerase, mediate disease pathogenesis, a hypothesis consistent with the fact that the median age at presentation for AML is 70.⁴⁰

Telomere shortening was significantly more pronounced in patients with cytogenetic alterations as compared with patients with normal karyotypes. In this study, the shortest median telomere length was found in the group with complex cytogenetic abnormalities. hTERT was overexpressed in patients with complex karyotypes, followed by patients with noncomplex karyotypes and patients without karyotypic changes.⁴¹ This might suggest that with increasing telomere attrition, by either replication-dependent or replication-independent mechanisms, karyotypic abnormalities becomes more pronounced and, as a consequence, telomerase upregulation becomes essential to prevent replicative senescence of the malignant clone. However, it has recently been suggested that telomerase expression in the context of short telomeres

does not necessarily prevent cells from reaching replicative exhaustion.⁴²

Because of the uneven distribution of telomere length on individual chromosome arms, critical shortening of telomeres on particular chromosomes could promote the formation of chromosomal aberrations and contribute to clonal evolution. This hypothesis remains relevant even if the average telomere length remains well above the critical level of shortening.⁴³ Distinct groups of AML that are characterized either by aberrations that could result from telomere dysfunction (terminal deletions, gains/losses of chromosome parts, or nonreciprocal translocations) or by aberrations that are unlikely to result from telomere dysfunction (e.g., reciprocal translocations or inversions) could serve as an ideal model to study the effect of telomere shortening and telomerase activity during tumorigenesis.⁴⁴

Chronic Myeloid Leukemia

Most probably due to an increased turnover of the BCR-ABL-positive haematopoietic compartment, myeloid cells from 123 patients with CML show accelerated telomere shortening. In Ph⁺ peripheral blood leukocytes, telomere length is approximately 1 kb shorter than in age-matched controls. Taking into account that roughly 100 bp (50–200 bp) are lost per cell division in somatic cells the reduced telomere length in CP CML cells indicates that, at a given point of time, leukaemic BCR-ABL-positive haematopoietic stem cells have undergone an excess of approximately 10 cell divisions as compared to their normal polyclonal counterparts (HSC). Furthermore, telomere length measurements of cells

obtained from CML patients suffering from different stages of disease showed significantly shorter telomeres in AP and BP than in CP as revealed by telomere fluorescence and Southern Blot analysis (reviewed in ref 43). Successful therapy with IM was found to be associated with an increase in mean telomere length (Figure 3) reflecting a treatment-induced shift from virtually 100% BCR-ABL+ peripheral blood cells to predominantly polyclonal, BCR-ABL haematopoiesis.^{46,47}

Clinical implication of telomere and telomerase roles in hematological disorders

Bone marrow failure syndrome

A telomerase mutation does not appear to be sufficient to determine aplastic anemia, but healthy relatives in which telomeres are short and/or a mutation need to be further observed in long-term prospective studies.²⁰ Besides this, protection from environmental stress should be minimized to prevent further hits for bone marrow failure development in acquired aplastic anemia. For most syndromes in which telomere shortening is known to be pathogenic, hematopoietic stem cell transplantation is the only potential cure. Modulation of telomerase activity may have a role in the treatment of telomere deficiency syndromes such as dyskeratosis congenita and telomerase-mutant acquired aplastic anemia. Clinical observations suggest that androgen therapy can induce improvements in peripheral blood counts, achieving transfusion independence in as many as 60% of patients.¹⁷ Androgens stimulate telomerase gene expression in hematopoietic cells, including CD34+ cells and lymphocytes, and this mechanism of action may explain their

efficacy in hematologic disease.⁴⁷ Androgen therapy may cause severe adverse events, such as hepatocarcinoma and peliosis hepatis, and liver function must be monitored. For patients with acquired aplastic anemia, hematopoietic stem cell transplantation and intensive immunosuppression with antithymocyte globulin and cyclosporine are the main therapies.²¹ Immunosuppressive therapy seems to be relatively or entirely ineffective for dyskeratosis congenita. Patients with acquired aplastic anemia and short telomeres appears to have poorer responses to immunosuppression. However, this relationship was retrospectively observed, and prospective studies of the prognostic value of telomere length are required before such measurements influence therapeutic decisions. Some patients with aplastic anemia with telomerase complex mutations respond hematologically to androgen treatment,⁴⁸ as do patients with dyskeratosis congenita.

Acute leukemia

Concepts of telomere and telomerase in acute leukemia are helpful for understanding the pathophysiology of the disease. Nevertheless, whatever the role of hTERT in determining the prognosis in AML, it is unlikely to surpass that of cytogenetics in discriminating risk groups yet.²⁰ In primary AML samples, the transfection with dominant negative hTERT reduced the number of colony forming units (CFU) and decreased engraftment of leukaemic cells transplanted into a immunodeficient mouse model.⁴⁹

Chronic myeloid leukemia

In the BCR-ABL+ CML cell line K562, 50% of the expanded clones underwent apoptosis after detectable telomere shortening

when transduced with the dominant negative form of hTERT.⁵⁰ Competitive inhibition of the catalytic activity of telomerase has been attained by the small molecule inhibitor BIBR1532 as well as by the oligonucleotide GRN163L both in vitro and in vivo experiments. However, in the BCR-ABL-positive K562 cell line, BIBR1532 mediated telomerase inhibition was insufficient to induce telomere mediated apoptosis, while the expression of a dominant negative hTERT mutant in the respective cell line induced an increased rate of apoptosis (mostly reflecting terminal 'crisis' cells) and augmented radiosensitivity.⁵⁰ GRN163L produced a decreased concentration of the compound required for telomerase inhibition and a better biodistribution in normal and malignant tissue.⁵¹

Transcriptional inhibition of essential telomerase components

Short interfering RNA (siRNA) causes the degradation of the corresponding mRNA in cells. In in vitro and in vivo experiments, telomerase activity was successfully downregulated by the introduction of siRNA against TERT. Antisense-oligodeoxynucleotides (ODNs), a short DNA fragment which is complementary to the respective target RNA, after hybridization with hTERT RNA, caused an inhibition of the RNA function and thus lead to a direct decrease of telomerase activity. Highly increased rates of apoptotic cells were observed in cell culture experiments with glioma, prostate carcinoma and ovarian cancer cell lines (reviewed in ref.44). It may be assumed that non- or slowly dividing somatic cells do not experience critical shortening of telomere length, while in fast growing malignant cells with a high telomerase activity, a critical telomere shortening can be

achieved earlier resulting in a mitotic catastrophe and consequently apoptosis and necrosis. However, it needs to be emphasized that an effective treatment will require time and effects will appear with delay which may potentially last from months to years, as shown in figure 4.

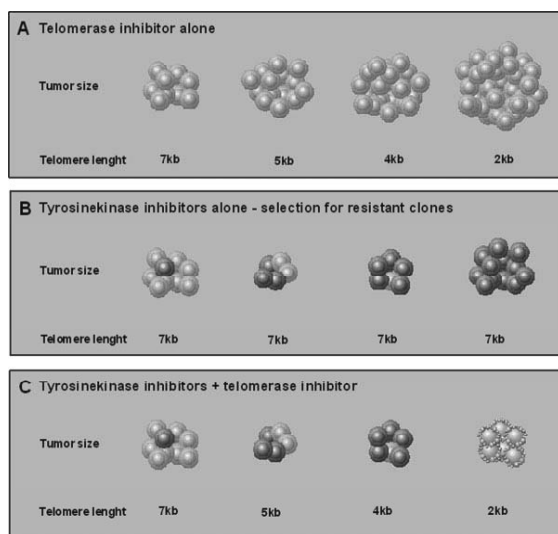


Figure 4. Simultaneous inhibition of telomerase and bcr-abl tyrosine kinase may overcome emergence of resistance. (A) Telomerase inhibitor alone: while telomeres shorten in response to treatment the malignant clone continues to proliferate. Depending on initial telomere length, telomeres become critically short after a distinct amount of time leading to cell senescence and apoptosis. (B) CML Phb cells are generally sensitive to tyrosine kinase inhibitors. However, secondary resistant subclones can emerge under therapy. (C) Combined treatment with tyrosine kinase and telomerase inhibitors represses the clonal expansion of tyrosine kinase resistant cells.⁴⁴

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