Telomere dynamics in adult hematological malignancies

Helena Olbertová a,b, Karla Plevová a,b, Kamila Stranska a,b, Sarka Pospisilova a,b

Telomeres are repetitive DNA sequences protecting physical ends of linear chromosomes against degradation and end-to-end chromosomal fusion. Telomeres shorten with each cell division, which regulates the cellular lifespan in somatic cells and limits their renewal capacity. Cancer cells are often able to overcome this physiological barrier and become immortal with unlimited replicative capacity. In this review, we present current knowledge on the role of telomeres in human aging with a focus on their behavior in hematological malignancies of adults. Associations of telomere length to age-related diseases and to the prevention of telomere shortening are also discussed.

Key words: telomere, telomerase, leukemia, lymphoma, hematooncology, aging

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*Center of Molecular Medicine, Central European Institute of Technology, Masaryk University, Brno, Czech Republic
bDepartment of Internal Medicine – Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Corresponding author: Sarka Pospisilova, e-mail: pospisilova.sarka@fnbrno.cz

Telomeres and their functions

Telomeres are complex repetitive DNA sequences binding multiple interacting proteins (Fig. 1), protecting the ends of linear chromosomes against end-to-end fusion and degradation. Human telomeres are composed of ∼5–15 kb long hexameric 5’-TTAGGG-3’ repeats. At the end of the chromosome, there is a 3’-overhang, which coils up into a T-loop and creates shelterin complex with telomere-binding proteins (Fig. 1). Telomeric DNA is transcribed into a non-coding telomeric repeat-containing RNA (TERRA) (ref.), which binds to the telomeric region creating DNA-RNA hybrids, and has a regulatory function. When a cell divides, the semiconservative DNA replication complex goes through many obstacles when approaching the telomeric regions. The replication fork needs the assistance of DNA helicases to resolve DNA-RNA hybrids and T-loops and the support of stability by shelterin proteins to successfully proceed through the telomeric region. Later, nucleases end-process the terminal nucleotides to generate the 3’-overhang. By this mechanism, the telomeres are shortened with each cell division. The telomeric region can be prolonged by an

![Fig. 1. A chromosomal end with telomeric repeats forming shelterin complex with T-loop that binds telomeric repeat binding factors 1 and 2 (TRF1 and TRF2), TRF1-interacting nuclear protein 2 (TIN2), human repressor activator protein 1 (RAP1) and single-stranded D-loop on 3’-overhang binding protection of telomeres 1 (POT1) and tripeptidyl peptidase 1 (TPP1).](image-url)
enzyme called telomerase, composed of the catalytic subunit TERT (telomerase reverse transcriptase) and RNA template telomerase RNA component (TERC) (ref.9,10). Another mechanism of telomere prolongation is called alternative lengthening of telomeres (ALT) based on homologous recombination. ALT is active in a minority of human cancer types and this underlies resistance to therapy based on anti-telomerase drugs11,12. It has been shown that TERRA levels are elevated in cells with active ALT (ref.13). Overall, telomere replication and prolongation include many complex processes and interactions; the detailed mechanism is not fully understood yet.

Telomere maintenance strategy is specific for each cell type. While telomeres of somatic cells shorten with each cell division, germ cells are capable of maintaining stable telomere length to sustain cell immortality14. In stem cells, telomerase activity is limited and during lifetime the proliferative capacity of these cells is exhausted, causing senescence and loss of ability to renew tissues15,16. However, many cancer cells are able to overcome short telomeres and activate telomerase to ensure cell immortality, thus increasing the aggressiveness of a tumor9.

Telomeres influence the health of aging individuals

Overall healthy lifestyle, including balanced diet, physical activities, and avoiding smoking and stress exposure, has great potential to reduce the rate of excessive telomere shortening and thus to delay the onset of age-associated diseases and increase lifespan17. Most unhealthy lifestyle factors have been linked to increased cellular oxidative stress18-21. Due to the high guanine content and a lack of repair of single-stranded breaks, telomeric regions are susceptible to damage caused by oxidative stress22, therefore reducing oxidative stress in cells results in better telomere maintenance. In many epidemiologic studies, short telomeres were associated with age-related diseases including cardiovascular diseases, osteoporosis, cancer, and overall mortality, although, probably due to different methodology and cohort selection, these results are often contradictory (epidemiologic studies reviewed in detail in Sanders and Newman23).

Telomere length in hematological malignancies of adults

Acute myeloid leukemia

Acute myeloid leukemia (AML) typically progresses rapidly with leukemic cells undergoing extensive cell division. Therefore, compared to age-matched healthy controls, short telomeres caused by gradual replicative erosion are frequently represented, with cytogenetically abnormal leukemic cells having shorter telomeres than leukemic cells with normal karyotype24,25. In AML, approximately half of the patients do not carry cytogenetic lesions at the time of diagnosis26,27; however, it was shown that 50% of patients with normal karyotype have submicroscopic losses or duplications in terminal regions suggesting impaired telomere stability28. AML cells with FLT3 mutations have shorter telomeres than FLT3 wild-type cells24. Telomere length differs among AML cases divided based on the French-American-British (FAB) classification system; with more differentiated cells the telomere length was shorter24. AML0 and AML3 subtypes express lower amount of TERT than other AML FAB subtypes25. Compared to less common adult acute lymphoblastic leukemia (ALL), AML patients have longer telomeres and lower expression of telomerase24,25.

Chronic myeloid leukemia

Most patients with chronic myeloid leukemia (CML) have no distinctive early disease symptoms as the disease typically evolves slowly in the chronic phase but later on progresses rapidly in the accelerated and blast phases. CML arises from cells with translocation t(9;22)(q34;q11), creating Philadelphia chromosome, and resulting in the BCR-ABL1 fusion gene with a high tyrosine kinase activity. CML patients have highly variable but generally shorter telomeres and normal or slightly higher expression of telomerase, than age-matched controls29,30. CML patients with shorter telomeres at the time of diagnosis represent a high-risk subgroup and enter the accelerated phase earlier18. Similarly, patients entering the blast phase within 2 years after diagnosis had significantly shorter telomeres31. Most of the studies focused on overall telomere length, while Samaesskou et al. studied telomere length of individual chromosomes in CML patients and found that parms harbored longer telomeres and the q-arms shorter because of high shortening rate on q-arms32. The telomere length on individual chromosomes differed greatly within the study; telomeres on 5p, 9p, 11p, 12p, 14p, 14q, 16p, 17p, 18p, 19p, 20p, Xp, and Xq were maintained or prolonged in most CML cases. Patients with a higher telomerase activity had a higher frequency of additional cytogenetic aberrations, in the blast phase the telomerase activity was increased compared to the chronic phase10. These findings suggest a potential association between individual telomere length and chromosomal (in)stability in CML.

Myelodysplastic syndrome

Myelodysplastic syndrome (MDS) is a highly heterogeneous disease with many subtypes harboring various genetic mutations. MDS originates from an undifferentiated hematopoietic stem cell and causes ineffective hematopoiesis, predisposition to infections, and frequently progresses to AML (ref.24). Risk factors for MDS include advanced age, previous exposure to chemotherapy, radiation, chemicals and some inherited genetic syndromes related to abnormalities in DNA repair. Compared to age-matched healthy controls, the MDS telomere length is shorter in peripheral blood leukocytes33,34 and even non-tumor buccal swabs35. There is no significant difference in telomere length between distinct MDS subtypes; however, patients with isolated monosomy 7 have significantly longer telomeres36. Shorter telomeres were detected in patients with professional exposure to paints and pesticides36. Overall, telomere dysfunction could be caused by deregulated RNA splicing and associates with high-risk MDS phenotypes38. Higher telomerase activity is associated with shorter survival in MDS patients39.
Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world with very heterogeneous features and disease course. Similarly to other indolent lymphoproliferative malignancies, at the time of diagnosis, CLL has none or ambiguous symptoms and many patients are diagnosed randomly. Patients divide into two main prognostic groups based on the somatic hypermutation status of their rearranged immunoglobulin heavy chain variable gene region (IGHV): mutated IGHV is associated with better prognosis, while unmutated IGHV with worse prognosis.40,41

Thoroughly studied in CLL, telomere length has been associated with markers of poor prognosis, such as unmutated IGHV, shorter time to first treatment, reduced overall survival, increased genomic complexity and mutated ATM and TP53 (ref.42-48).

Telomere length is considered to be stable in the disease course of most CLL cases49, although our recent unpublished data suggest that due to clonal evolution, telomere length might vary under specific circumstances.

Telomerase activity in CLL has been shown to be significantly higher in IGHV-unmutated cells compared to IGHV-mutated cells42,50. Despite this, the telomerase activity in CLL was similar to normal B lymphocytes and was not shown to associate with patients’ prognosis42. In contrast, other studies show a significant association between telomerase activity and survival in CLL (ref.49,50). Of note, IGHV-mutated CLL originates from cells activated in germinal center (GC) where telomeres in normal B cells are naturally prolonged by telomerase. In contrast, the IGHV-unmutated CLL cells originate likely at the pre-GC developmental stage where telomeres are naturally shorter51. Discrepancies in the activity of telomerase might be caused by the complexity of telomere maintenance and the role of shelterin and other proteins in this process. Mutations in POT1, an important member of the shelterin complex maintaining 3'-overhang stability, have been described in 3.5% of IGHV-mutated, and 9% of IGHV-unmutated cases53. Expression levels of telomerase maintenance proteins TRF1, RAP1, and POT1 were shown to be reduced and levels of TPP1 increased74 in newly diagnosed CLL patients at an early stage; TPP1 and TIN2 were down-regulated65. Expression of other proteins interacting with telomeres remains to be investigated further.

Non-Hodgkin lymphomas

Non-Hodgkin lymphomas (NHL) are the most common malignancies among adult hemato-oncological patients. They originate from B lymphocytes at various developmental stages, less frequently NHL can arise from T lymphocytes. In this review, we will focus on the most common types of NHL diagnosed in Europe.

Diffuse large B cell lymphoma

Diffuse large B cell lymphoma (DLBCL) is the most common NHL characterized by rapid cell division and fast growth of lymph nodes. Typically, treatment is immediately needed and induces various responses in different disease subtypes. DLBCL cells originate from GC B cells. Two main subgroups are GC-like DLBCL with ongoing mutagenesis and better prognosis and activated B cell-like (ABC) DLBCL with poor prognosis46. At molecular level, DLBCL harbors a large number of aberrations, often including del(1p), del(2p), and del(13q) in GC-like DLBCL and 3+, del(6q), del(9p), gain 18q, and gain 19q in ABC-DLBCL (ref.57). Like CLL, telomere length of both DLBCL subgroups corresponds to cellular origin. Thus GC-like DLBCL originating from GC has longer telomeres than post-GC ABC-DLBCL (ref.58). GC-like DLBCL and ABC-DLBCL have different overall survival; however, telomere length detected in the subgroups does not further separate patients according to overall survival59.

Mantle cell lymphoma (MCL)

Mantle cell lymphoma (MCL) has intermediate cell growth and high clinical and genetic heterogeneity with typical recurrent genomic aberrations including t(11;14) (q13;q32) juxtaposing CCND1 gene in a close proximity of IGH locus which leads to cyclin D1 overexpression. Gene mutations in ATM, TP53, CCND1, and NOTCH1 are used as markers of prognosis5961. Genomic instability expressed by various genomic alterations in MCL suggests a possible role of telomere dysfunction. Telomere length in MCL is shorter than in normal B cells, DLBCL, and FL. Overall MCL cells have longer telomeres than IGHV-unmutated CLL cells but shorter than IGHV-mutated CLL cells55. No significant association of telomere length with IGHV somatic hypermutation, TP53 mutations, proliferation index, or overall survival was found59,62.

Follicular lymphoma (FL)

Follicular lymphoma (FL) is an indolent lymphoma with slow cell growth, where the watch and wait approach is often employed. FL cells originate from GC, 90% of cases carry typical t(14;18)(q32;q21) juxtaposing BCL2 gene in a close proximity of the IGH locus causing overexpression of anti-apoptotic proto-oncogene BCL2 (ref.63). Telomere length in FL is similar to DLBCL but longer than in MCL (ref.64).

Overall, in NHLs, telomerase is naturally active following a cellular origin based on GC activation and is not overexpressed compared to normal reactive lymph node tissues; overexpression was detected only in Burkitt’s lymphoma52,65. In DLBCL, MCL, and FL, a constantly low level of telomerase activity was noted, although proliferation levels in these NHLs vary. In normal lymph nodes, DLBCL, MCL, and FL, the expression of TRF1, TRF2, and PIF1 was reported to differ only marginally65. Since no other information about telomere maintenance strategy in NHLs has been found, this field appears relatively unexplored.

Hodgkin lymphoma

Hodgkin lymphoma (HL) is a malignancy featuring a low number of malignant cells in affected lymph nodes, consisting of multi-nucleated Reed–Sternberg cells and their precursors, mono-nucleated Hodgkin cells, derived
from GC-B cells\textsuperscript{66}. Short telomeres were found in both malignant cells and non-tumor blood cells compared to healthy donors\textsuperscript{67}. Patients with refractory disease have shorter telomeres even before first treatment administration\textsuperscript{68}. Short telomeres in HL are associated with increased genomic instability\textsuperscript{67}. Telomerase is expressed in low levels in some cases but it is not detectable in other cases\textsuperscript{69,70}. In HL the telomeres can be prolonged by both telomerase and ALT as well. The occurrence of ALT is associated with disease progression and shorter overall survival in HL (ref.\textsuperscript{71}).

Multiple myeloma

Multiple myeloma (MM) originates from antibodies-producing GC-experienced plasma cells. MM evolves from a mostly asymptomatic condition known as monoclonal gammopathy of undetermined significance (MGUS) (ref.\textsuperscript{72}) and in an advanced stage is typically characterized by severe bone pain caused by osteolytic skeletal lesions\textsuperscript{73}. MM is very heterogeneous at the molecular level with 40% of cases characterized by trisomies and with most of the remaining cases having a translocation involving IGH locus on chromosome 14q32 or deletion of 13q14 (ref.\textsuperscript{74,75}). The presence of aberrations influences prognosis and therapy response\textsuperscript{76}. Whole genome sequencing of MM samples revealed multiple mutations in genes belonging to the NF-κB pathway and genes involved in protein translation and histone methylation\textsuperscript{77}. Telomere length in MM is shorter than in MGUS and displays heterogeneity among patients\textsuperscript{78}. Shorter telomere length and higher telomerase activity correlate with poor prognostic features in MM (ref.\textsuperscript{78,79}). In particular, telomerase activity is a strong predictor of 1- and 2-year survival\textsuperscript{79}. It has been shown in \textit{in vitro} experiments that telomerase inhibition could represent a promising treatment option in MM (ref.\textsuperscript{80}).

**CONCLUSION**

Telomeres are protective structures at the ends of linear chromosomes. They are naturally shortened within each cell division causing senescence after a large number of divisions; telomere maintenance is a complex process. Telomere length, expression of telomerase and activity of telomere-related proteins are more or less associated with various cancer types including hematological malignancies of adult age. The role of telomeres and their maintenance have been studied in individual hematological malignancies (Table 1) using various approaches. Unfortunately, the accuracy of the methods used is open to question. Repetitive telomeric regions are difficult to approach and it is challenging to collect reproducible results even with a single assay. Therefore, although the diagnostic use of telomere length could have validity in prognostication, it is currently not usually used, as other disease-specific markers are more accessible. In some malignancies, the results can be significantly influenced by the presence of non-tumor cells in an analyzed sample. Overall, it would be valuable to conduct a comparative study across hematological malignancies studying telomere length, telomerase activity, and telomere-associated proteins and genes, with the use of reproducible approaches, samples with high tumor cell content and a sufficient number of samples per each disease entity.

**Search strategy and selection criteria**

English-written original scientific articles and reviews from 1980 to 2018 were searched using PubMed and Google Scholar.

Key words for the search were as follows: “telomere length in leukemia”, “telomere length in lymphoma”, “telomere structure”, “telomere replication”, “telomere maintenance”, “telomeres and epidemiology”, “telomeres and oxidative stress”, “telomere shortening prevention”.

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**Table 1.** Comparison of telomere length and telomerase activity and their association with disease prognosis in hematological malignancies.

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Telomere length comparison</th>
<th>Telomere length associated with prognosis</th>
<th>Telomerase activity associated with prognosis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>&gt; ALL = CML; &lt; MDS</td>
<td>yes</td>
<td>yes</td>
<td>25,81</td>
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<td>CML</td>
<td>= AML; &lt; MDS</td>
<td>yes</td>
<td>yes</td>
<td>81</td>
</tr>
<tr>
<td>MDS</td>
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<td>yes</td>
<td>81</td>
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<tr>
<td>CLL</td>
<td>= MCL; &lt; DLBCL, FL</td>
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<tr>
<td>DLBCL</td>
<td>&gt; CLL, MCL; = FL</td>
<td>no</td>
<td>no</td>
<td>58</td>
</tr>
<tr>
<td>MCL</td>
<td>= CLL; &lt; DLBCL, FL</td>
<td>no</td>
<td>no</td>
<td>58</td>
</tr>
<tr>
<td>FL</td>
<td>&gt; CLL, MCL; = DLBCL</td>
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<td>no</td>
<td>58</td>
</tr>
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<td>HL</td>
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<td>yes</td>
<td>67</td>
</tr>
<tr>
<td>MM</td>
<td>&lt; MGUS</td>
<td>yes</td>
<td>yes</td>
<td>25</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma.
ABBREVIATIONS
ABC, activated B cell like; ABL1, ABL proto-oncoprotein 1 non-receptor tyrosine kinase; ALL, acute lymphoblastic leukemia; ALT, alternative lengthening of telomeres; AML, acute myeloid leukemia; ATM, ATM serine/threonine kinase; BCL2, BCL2 apoptosis regulator; BCR, breakpoint cluster region; CCND1, cyclin D1; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B cell lymphoma; DNA, deoxyribonucleic acid; FAB subtypes, French-American-British subtypes; FL, follicular lymphoma; FLT3, fms related tyrosine kinase 3; GC, germinal center; HL, Hodgkin lymphoma; IGH, immunoglobulin heavy chain variable region; MCL, mantle cell lymphoma; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NHEJ, non-Hodgkin lymphoma; NOTCH1, notch 1; P53, tumor protein p53; POT1, protection of telomeres 1; RNA, ribonucleic acid; RAP1, repressor activator protein 1; RER, reverse end replication; TERT, telomerase reverse transcriptase; TIN2, TRF1-interacting nuclear protein 2; TPP1, tripeptidyl peptidase 1; TRF1, telomeric repeat binding factor 1; TRF2, telomeric repeat binding factor 2.

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