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Telomere shortening and ageing

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Telomerverkürzung und Alterung

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Abstract Telomeres form the ends of human chromosomes. Telomeres shorten with each round of cell division and this mechanism limits proliferation of human cells to a finite number of cell divisions by inducing replicative senescence, differentiation, or apoptosis. Telomere shortening can act as a tumor suppressor. However, as a downside, there is growing evidence indicating that telomere shortening also limits stem cell function, regeneration, and organ maintenance during ageing. Moreover, telomere shortening during ageing and disease is associated with increasing cancer risk. In this review we summarize our current knowledge on the role of telomere shortening in human ageing, chronic diseases, and cancer.

Key words telomeres – ageing – cancer – chronic disease – replicative senescence

Zusammenfassung Telomere bilden die Endstücke menschlicher Chromosomen und sind für den Erhalt der Chromosomenstabilität essentiell. Telomere verkürzen sich aber mit jeder Zellteilung, wodurch die proliferative Lebesspanne menschlicher Zellen limitiert wird, da bei einer kritischen Verkürzung der Telomere Seneszenz, Stammzelldifferenzierung oder Apoptose induziert wird. Diese Telomer-abhängige Begrenzung der Lebensspanne menschlicher Zellen wird allgemein als Tumorsuppressormechanismus angesehen. Es gibt aber zunehmend Hinweise, dass die Verkürzung der Telomere auch nachteilige Auswirkungen im Rahmen der Alterung haben kann, da hierdurch die Funktion von Stammzellen und die regenerative Reserve der Organe beschränkt wird. Darüber hinaus ist die Verkürzung der Telomere im Rahmen der menschlichen Alterung und bei chronischen Erkrankungen mit einem erhöhten Tumorrisiko assoziiert. In dieser Übersicht fassen wir unser aktuelles Wissen über die Rolle von Telomeren in der Alterung, bei chronischen Erkrankungen und hinsichtlich der Krebsentstehung zusammen.

Schlüsselwörter Telomere – Altern – Krebs – Chronische Erkrankungen – Replikative Seneszenz

Telomeres and telomerase

Telomeres consist of simple tandem DNA repeats that do not encode for any gene product [1]. The main function of telomeres is to cap the chromosome ends [1]. Telomere capping is necessary to distinguish the chromosome ends from DNA breaks within the genome. A DNA break within the genome leads to cell cycle arrest and DNA repair or to induction of apoptosis when the damage is too severe or non-repairable. In contrast to DNA breaks, the chromosome ends should not provoke DNA damage responses. Telomeres prevent the induction of such responses at the chromosome termini and this capping is a prerequisite to maintain chromosome stability. To fulfil capping function telomeres need to have a minimum length. In addition, telomeres form 3-dimensonal structures (T-loops), which are required for telomere capping [2]. Telomere binding proteins represent a group of proteins that bind with high specificity at the telomeres [2]. These proteins are required for the stabilization of the telomere structure and telomere capping [2].

The problem with telomeres is that telomeres shorten with each round of cell division [3]. Telomere shortening is due to the "end replication problem" of DNA-polymerase – this enzyme cannot fully replicate the very end of linear DNA during the S-phase of the cell cycle [4]. Additional factors (processing of telomeres during the cell cycle, reactive oxygen species) can contribute to telomere shortening [4, 5].

To compensate telomere shortening an enzyme exists that can synthesize telomere *de novo*. This enzyme is called telomerase and consists of two essential components: (i) the telomerase RNA component (Terc) is a functional RNA that serves as a template for telomere sequence synthesis, (ii) the telomerase reverse transcriptase (Tert) is the catalytic subunit of the enzyme [6]. Both components are essential for telomerase activity. In humans, telomerase is active during embryogenesis, but is suppressed postnatally in most somatic tissues. In adult humans, telomerase stays active only in germ cells and certain stem cell and progenitor compartments. In addition, telomerase reactivation occurs in activated lymphocytes and human cancer (see below).

Telomere shortening and cellular ageing

Telomeres shorten in human cells with each round of cell division, because of the "end replication problem" of DNA polymerase and because of the suppression of telomerase expression (see above). In humans, fibroblast telomeres shorten 50–100 basepairs with each cell division [3]. When telomeres reach a critically short length they lose the capping function at the chromosome termini. These dysfunctional telomeres are then recognized as DNA damage and induce DNA damage checkpoints [7]. Two checkpoints have been identified that limit cellular lifespan in response to telomere dysfunction: The first checkpoint (first mortality stage = M1) is characterized by a permanent cell cycle arrest [8]. This checkpoint is called senescence and depends on activation of the tumor suppressor gene p53. Human fibroblasts enter senescence after 50–70 cell divisions [3]. Cells with mutant p53 can bypass the senescence checkpoint and continue to proliferate despite the presence of critically short, dysfunctional telomeres. However, ongoing telomere shortening leads to a further increase in telomere dysfunction inducing a second checkpoint (second mortality stage = M2), which is called crisis. Crisis is p53-independent and is characterized by massive chromosomal instability and cell death [8]. Telomere shortening, senescence and crisis limit the replicative lifespan of primary human cells, thus, acting as a tumor suppressor mechanism limiting the lifespan of transformed cells [8]. As a downside, telomere dysfunction and checkpoint responses could contribute to the exhaustion of cellular function and impaired organ maintenance during ageing.

Telomere shortening during human ageing

Most human tissues and organs show significant telomere shortening during ageing [9] including peripheral blood cells, lymphocytes, kidney epithelium, vascular endothelial cells, hepatocytes, intestinal epithelial cells, lung epithelial cells, muscle, and others (Table 1) [9].

There are a view organs and tissues which do not show significant telomere shortening during ageing: (i) The human brain did not show significant telomere shortening during ageing when whole organ biopsies were analyzed [10]. However, it is unknown whether certain cell subpopulation within the human brain (e.g. human neuronal stem cells) experience telomere shortening during ageing. In addition, it is possible that other cell types, which experience telomere shortening, affect brain function during ageing. Along these lines it has been shown that patients with Alzheimer disease have abnormal short telomere in lymphocytes compared to non-Alzheimer controls [11]. Moreover, short telomeres in peripheral blood correlate with poor outcome after brain infarction [12]. (ii) There are contrasting re-

Table 1 Human ageing and telomere length

Organ	Cell type	Telomere length change	Publication
Kidney	Kidney tissues	Significantly shortened	Gardner JP et al., 2007
	The inner medulla, the outer renal cortex	Significantly shortened	Tchakmakjian L et al., 2004
	The outer renal cortex	Significantly shortened	Melk et al., 2000
	Tissue and sections	Significantly shortened	Ferlicot S et al., 2003
	Renal cortex	Significantly shortened	Takubo K et al., 2002
Cardiovascular system	Aorta tissues	Significantly shortened	Okuda K et al., 2000
	Vascular endothelial cells	Significantly shortened	Furumoto K et al., 1998
Liver	Liver tissue	Significantly shortened	Takubo K et al., 2000, 2002
	Hepatocytes	Significantly shortened	Wiemann SU et al., 2002
	Tissue and sections	Significantly shortened	Ferlicot S et al., 2003
	Liver tissue	Significantly shortened	Paradis V et al., 2001
	Liver tissue	Significantly shortened	Aikata H et al., 2000
Colon	Colorectal tissue	Significantly shortened	O'Sullivan J et al., 2006
	Mucosal biopsies	Significantly shortened	O'Sullivan J et al., 2004
	Mucosal biopsies	Significantly shortened	Hastie ND et al., 1990
Stomach	Gastric mucosal specimen	Significantly shortened	Furugori E et al., 2000
Esophagus	Mucosal biopsies	Significantly shortened	O'Sullivan J et al., 2004
Spleen	Spleen tissue	Significantly shortened	Takubo K et al., 2002
Blood	Hematopoietic stem cells	Significantly shortened	Vaziri H et al., 1994
	Lymphocytes	Significantly shortened	Vaziri H et al., 1993
	Peripheral blood cells	Significantly shortened	Slagboom PE et al., 1994
Lung	Lung tissues	Significantly shortened	Gardner JP et al., 2007
Skin	Whole thickness biopsy	Significantly shortened	Gardner JP et al., 2007
	Epidermis	Significantly shortened	Sugimoto M et al., 2006
Skeletal muscle	Skeletal muscle tissues	Significantly shortened	Gardner JP et al., 2007
Thyroid	Thyroid tissues	Significantly shortened	Kammori M et al., 2002
Pancreas	Whole organ biopsy	Significantly shortened	Ishii A et al., 2006
Brain	Cerebral cortex	No difference	Takubo K et al., 2002
Cardiovascular system	Myocardium	No difference	Takubo K et al., 2002

ports on telomere shortening in human skin [13, 14]. However, there is an accumulation of senescent cells in ageing human skin [15, see below]. A possible explanation is that there are subclones with critically short telomere in human skin, but these clones are not detectable when telomere length is determined in whole skin biopsies. (iii) In agreement with high levels of telomerase expression, human germ cells maintain long telomeres during ageing [16]. In contrast, there is evidence that telomeres shorten in adult stem cells during human ageing, despite low levels of telomerase expression in these cells [17]. A current model indicates that telomerase expression in stem cells is necessary for the high proliferative capacity of these cells, but the level of telomerase is not sufficient to maintain telomeres stable in stem cells during a lifetime [18]. According to this hypothesis it is conceivable that telomere shortening contributes to the age-associated decline in adult stem cell function [19].

In addition to telomere shortening in most human tissues during ageing, there is some evidence for an accumulation of senescent cells (exhibiting senescence associated β-galactosidase activity = SA-βGal) in some human tissue during ageing, e.g. skin fibroblast [15]. SA-βGal positive cells have also been observed in human liver cirrhosis – the end stage of chronic liver disease [20]. However, the accumulation of senescent cells in ageing human tissues appears to be low compared to the strong evidence for telomere shortening in almost all human tissues during ageing (see above). A possible explanation is that telomere shortening in human tissues is not sufficient to induce a widespread accumulation of senescent cells. Alternatively, senescent cell might be removed from ageing organs. Along these lines, it has been shown that senescent cells can be removed by immune responses and apoptosis [21, 22].

There is emerging evidence that telomere shortening has functional impact on ageing and ageing

associated disease. Telomere length in the peripheral mononuclear cells correlated inversely with the mortality rate in humans 60–75 years old [23]. Individuals with short telomeres had a 3.18-fold higher mortality rate from heart diseases, and 8.54-higer mortality rates from infectious diseases compared to those with relatively long telomeres. Other studies have confirmed the connection between telomere shortening and the evolution of cardiac disease [24, 25]. However, telomere shortening was not a significant prognostic factor for survival in humans 85 years and older [26]. These data indicate that in the oldest old, other factors than telomere length are determining lifespan.

Telomere shortening during diseases

In addition to telomere shortening during ageing, accelerated telomere shortening occurs in various human diseases (Table 2). Telomere shortening has been observed in lymphocytes during chronic HIV infection, in hepatocytes during chronic hepatitis [20], in intestinal epithelium of patients with chronic inflammatory bowel disease, in various forms of anemia, in lymphocytes of patients with Alzheimer disease [11], in vascular endothelial cells of artherosclerotic plaques, and in other diseases (for review see Table 2). In addition, telomere shortening appears to correlate with disease progression in some diseases. One example is liver cirrhosis, which evolves at the end-stage of chronic liver disease. A current model indicates that chronic hepatocyte damage leads to increased hepatocyte turn over and accelerated telomere shortening in this compartment [20]. Critically short telomeres will then lead to hepatocyte senescence promoting the evolution of liver cirrhosis characterized by decreased regenerative reserve, fibrotic scarring and organ failure [20]. In agreement with this model, telomere shortening correlates with the evolution and progression of liver cirrhosis [20]. Another example is a hematopoietic disorder, which is called myelodysplastic syndrome (MDS). MDS is an ageing associated disease – a clonal hematopoietic stem cell disorder – leading to bone marrow failure [27]. Telomere shortening occurs in blood cells of MDS patients and correlates with disease progression [28]. In addition, telomere length and/or telomerase activity have been suggested as markers for the initiation and progression of various human tumors including cancers in liver, lung, breast, prostate, colon, brain, pancreas and head and neck, as well as malignancies of the hematopoietic system (see Table 2).

Experimental evidence for a role of telomere shortening in organismal ageing

Studies in telomerase deficient mice have provided first experimental evidence that telomere shortening can impair organ maintenance and lifespan. Telomerase deficient mice carry a homozygous deletion of the RNA component of telomerase (mTerc–/–) [29] or of the catalytic subunit of the enzyme (Tert–/–). Both knockouts lack telomerase activity and show similar phenotypes in response to telomere shortening. Terc–/– mice with short telomeres showed premature ageing phenotypes, specifically affecting organ systems with high rate of cell turnover [21, 30]. Accelerated ageing in these mice correlated with the increased frequency of cells with critically short telomeres and chromosomal free ends. Ageing telomere dysfunctional mice show an atrophy of the intestinal epithelium, an atrophy of the splenic white pulp, impaired B-lymphopoiesis, as well as a reduction in the number and function of hematopoietic stem cells [21, 30]. Moreover, the mice have a reduced capacity of organ regeneration [31] and a reduced stress response (impaired wound healing, reduced hematopoiesis in response to chemotherapy) [30].

Experiments in mTerc–/– mice revealed that both cell intrinsic checkpoints and cell extrinsic alterations of the environment contribute to the decline in stem cell function in response to telomere dysfunction and ageing [21, 32]: The cell cycle inhibitor p21 is a downstream target of p53 inducing cell cycle arrest in response to DNA damage. Studies on human fibroblasts have shown that upregulation of p21 induces cellular senescence in response to telomere dysfunction [33]. Deletion of p21 prolonged the lifespan of Terc–/– mice without promoting the cancer formation. The increased lifespan of p21–/–, Terc–/– mice correlated with an improvement in stem cell function and organ maintenance [21]. These experiments provided a proof of principle that cell intrinsic checkpoints can limit stem cell function and organismal lifespan in response to telomere dysfunction. Moreover, these studies indicate that the inhibition of cell intrinsic checkpoints can be evaluated as a therapeutic target for the treatment of ageing associated organ degeneration. In addition to the role of cell intrinsic checkpoint, there is emerging evidence that telomere dysfunction induces cell extrinsic alteration limiting stem cell function [32]. Telomeres dysfunction in mTerc–/– mice was associated with a reduction in number and function of bone marrow stromal cells supporting the maintenance of HCS. Ageing telomere dysfunctional mice also develop an aberrant expression of cytokines and growth factors in blood serum associated with impaired B-lympho-

Table 2 Telomere length and human disease

Disease	Cell type	Telomere length change	Publication
Aplastic anaemia (AA)	PBMCs	Significantly shortened	Ball SE et al., 1998
	PBMCs	Significantly shortened	Lee JJ et al., 2001
	PBMCs	Significantly shortened	Brümmendorf TH et al., 2001
	Granulocyte	Significantly shortened	Calado RT et al., 2007
Fanconi anaemia (FA)	Hematopoietic cells	Significantly shortened	Franco S et al., 2004
	PB lymphocytes	Significantly shortened	Hanson H et al., 2001
	PB lymphocytes	Significantly shortened	Leteurtre F et al., 1999
	PB lymphocytes	Significantly shortened	Li X et al., 2003
	PB lymphocytes	Significantly shortened	Callén E et al., 2002
	PB lymphocytes	Significantly shortened	Calado RT et al., 2004
Autosomal dominant dyskeratosis congenita (ADDC)	Whole blood	Significantly shortened	Vulliamy T et al., 2002
	BM	Significantly shortened	Vulliamy T et al., 2004
	BM	Significantly shortened	Marrone A et al., 2005
	PB lymphocytes	Significantly shortened	Knudson M et al., 2005
	PB cells	Significantly shortened	Goldman F et al., 2005
	PB cells	Significantly shortened	Armanios M et al., 2005
	Stem cells	Significantly shortened	Vulliamy T et al., 2006
	Total lymphocytes, CD45RA-positive, CD20-negative naive T-cells, and CD20-positive B-cells lymphocyte	Significantly shortened	Alter BP et al., 2007
X-linked dyskeratosis congenita	Lymphoblastoid cell lines derived from patients with X linked DC	Significantly shortened	Montanaro L et al., 2003
	PB cells	Significantly shortened	Vulliamy TJ et al., 2001
	Primary dermal fibroblast	Significantly shortened	Mitchell JR et al., 1999
	Primary fibroblasts and lymphoblasts	Significantly shortened	Wong JM et al., 2006
Hoyeraal-Hreidarsson syndrome (HHS)	PBl lymphocytes and fibroblasts	Significantly shortened	M'kacher R et al., 2003
Myelodysplastic syndrome (MDS)	BM or PB mononuclear cells	Significantly shortened	Ohyashiki JH et al., 1999
	BM	Significantly shortened	Ohyashiki JH et al., 1994
	BM or PB mononuclear cells	Significantly shortened	Sashida G et al., 2003
	BM and PB	Significantly shortened	Sieglová Z et al., 2004
	BM and PB	Significantly shortened	Ohyashiki K et al., 2005
	Granulocyte, CD14(+) and CD3(+) subpopulations obtained from bone marrow and peripheral blood	Significantly shortened	Terasaki Y et al., 2002
	Granulocytes	Significantly shortened	Cermák J et al., 2005
	BM or the granulocyte and lymphocyte cell	Significantly shortened	Boultwood J et al., 1997
	PB	Significantly shortened	Fern L et al., 2004
	BM	Significantly shortened	Bhatia R et al., 2005
Mature B-cell lymphoproliferative disorders	B-cell	Significantly shortened	Ladetto M et al., 2004
Shwachman-Diamond syndrome	Granulocyte	Significantly shortened	Calado RT et al., 2007
Paroxysmal nocturnal hemoglobinuria (PNH)	PB granulocytes	Significantly shortened	Beier F et al., 2005
Alzheimer's disease (AD)	PBMCs	Significantly shortened	Panossian LA et al., 2003
	PBMCs	Significantly shortened	Flanary BE et al., 2007
Atherosclerosis	Segments of the abdominal aorta	Significantly shortened	Okuda K et al., 2000
Atherosclerosis	PBMCs	Significantly shortened	Yamada N, 2003
	PBMCs	Significantly shortened	Adaikalakoteswari A et al., 2005
Atherosclerosis	White blood cells, and carotid artery atherosclerotic plaques	Significantly shortened	Benetos A et al., 2004
Atherosclerosis	Vascular smooth muscle cells (VSMCs) in atherosclerotic plaques	Significantly shortened	Matthews C et al., 2006

Table 2 (continued)

Disease	Cell type	Telomere length change	Publication
Cardiac hypertrophy	Vascular smooth muscle cells	Significantly shortened	Balasubramanyam M et al., 2007
Cardiovascular disease (CVD)	White blood cells	Significantly shortened	Nakashima H et al., 2004
	Leukocytes	Significantly shortened	Epel ES et al., 2006
	Leukocytes	Significantly shortened	Fitzpatrick AL et al., 2007
Coronary artery disease (CAD)	PB leukocytes	Significantly shortened	Samani et al., 2001
	PB leukocytes	Significantly shortened	Nowak R et al., 2002
	Leukocyte	Significantly shortened	Samani NJ et al., 2001
	Leukocyte	Significantly shortened	Matsubara Y et al., 2006
	PBMCs	Significantly shortened	Obana N et al., 2003
	Coronary endothelial cells	Significantly shortened	Ogami M et al., 2004
	WBCs	Significantly shortened	Balasubramanyam M et al., 2007
Coronary disease with metabolic disorders	PBMCs	Significantly shortened	Obana N et al., 2003
Coronary heart disease	Leukocytes	Significantly shortened	Brouilette SW et al., 2007
Calcific aortic valve stenosis (CAS)	Leukocytes	Significantly shortened	Kurz DJ et al., 2006
Chronic heart failure	Leukocytes	Significantly shortened	van der Harst P et al., 2007
End-stage heart failure	Endothelial cells	Significantly shortened	Balasubramanyam M et al., 2007
Premature myocardial infarction (MI)	White blood cell	Significantly shortened	Brouilette S et al., 2003
Chronic ulcerative colitis	Tissue sections from	Significantly shortened	Kinouchi Y et al., 1998
	histologically normal mucosa	Significantly shortened	O'Sullivan JN et al., 2002, 2004
Inflammatory bowel disease	Lymphocyte	Significantly shortened	Risques RA et al., 2006
Crohn disease	Intestinal intraepithelial lymphocytes (IEL) from the inflamed ileal mucosa	Significantly longer	Meresse B et al., 2001
	vs. IEL in the uninvolved ileal mucosa, IEL from Crohn disease Vs. control	Significantly shortened	Meresse B et al., 2001
Li Fraumeni Syndrome (LFS)	PB lymphocyte	Significantly shortened	Tabori U et al., 2007
Hypertension and diabetes mellitus	WBCs	Significantly shortened	Balasubramanyam M et al., 2007
Type 1 insulin-dependent diabetes (IDDM)	WBCs	Significantly shortened	Jeanclos E et al., 1998
Type 2 diabetes	Leucocyte	Significantly shortened	Adaikalakoteswari A et al., 2005
	Leukocyte	Significantly shortened	Adaikalakoteswari A et al., 2007
	Peripheral venous monocyte	Significantly shortened	Sampson MJ et al., 2006
Impaired glucose tolerance (IGT)	Leukocyte	Significantly shortened	Adaikalakoteswari A et al., 2007
Chronic gastroesophageal reflux disease	Esophageal squamous epithelium	Significantly shortened	Souza RF et al., 2007
Ataxia telangiectasia	T lymphocytes	Significantly shortened	Metcalfe JA et al., 1996
Down syndrome (DS)	T lymphocytes	Significantly shortened	Jenkins EC et al., 2006
	Progenitor/stem cell	Significantly shortened	Holmes DK et al., 2006
Duchenne muscular dystrophy (DMD)	Dystrophic tissue	Significantly shortened	Decary S et al., 2000
HIV type 1 infection	CD8(+) T cell	Significantly shortened	Wolthers KC et al., 1997
HIV	CD28-CD8+ T cell	Significantly shortened	Effros RB et al., 1996
	CD4+ T-lymphocyte	Significantly shortened	Nichols WS et al., 1999
	Blood mononuclear cells (BMNC) and purified CD8+ and CD4+ cells	Significantly shortened	Søndergaard SR et al., 2002
	Cord blood leukocytes and PB leukocytes	Significantly shortened	Poirier MC et al., 2003
	CD8(+) T cell	Significantly shortened	Dagarag M et al., 2003
	CD8(+) T cell	significant shortened	Dagarag M et al., 2004

Table 2 (continued)

Disease	Cell type	Telomere length change	Publication
	PBMCs (CD4+, CD8+, B cells)	Significantly shortened	Pommier JP et al., 1997
	PBMCs	Significantly shortened	Zeichner SL et al., 1999
	PBMCs	Significantly shortened	Feng YR et al., 1999
	PBMCs, CD4+ T-cell	Significantly shortened	Richardson MW et al., 2000
	Total PBMCs, CD4+ and CD8+ cells	Significantly shortened	Bestilny LJ et al., 2000
	T lymphocytes	Significantly shortened	Sidorov IA et al., 2004
Rheumatoid arthritis	Peripheral CD4 T cell	Significantly shortened	Wagner UG et al., 1998
	PB blood HLA-DR4+ cells	Significantly shortened	Schönland SO et al., 2003
	PB leukocytes	Significantly shortened	Pedersen-Lane JH et al., 2007
	WBCs	Significantly shortened	Steer SE et al., 2006
Chronic renal allograft rejection	Tubular epithelial cells	Significantly shortened	Joosten SA et al., 2003
Wegener's granulomatosis	T cells	Significantly shortened	Vogt S et al., 2003
Renal ischemia/reperfusion (I/R)	Renal cells	Significantly shortened	Chkhotua A et al., 2006
Ischaemia/reperfusion of donor kidney	Renal cells	Significantly shortened	Chkhotua AB et al., 2005
IgA nephropathy	PBMCs, urinary sediment	Significantly shortened	Szeto CC et al., 2005
Systemic lupus erythematosus (SLE)	CD4+, CD8+ and CD19+ lymphocytes	Significantly shortened	Lin J et al., 2005
	CD4+ T cell	Significantly shortened	Fritsch RD et al., 2006
	PBMCs	Significantly shortened	Honda M et al., 2001
	PBMCs	Significantly shortened	Kurosaka D et al., 2003
	Peripheral polymorphonuclear-neutrophils (PMN), mononuclear cells (MNC)	Significantly shortened	Wu CH et al., 2007
	T and B lymphocytes, PMN	Significantly shortened	Kurosaka D et al., 2006
Chronic hepatitis B virus (HBV)	PB lymphocytes	Significantly shortened	Satra M et al., 2005
Chronic hepatitis C virus infection (HCV)	PB lymphocytes	Significantly shortened	Satra M et al., 2005
	whole liver biopsy	Significantly shortened	Sekoguchi S et al., 2007
Chronic hepatitis (HBV & HCV)	Whole liver biopsy	Significantly shortened	Kitada T et al., 1995
	Whole liver biopsy	Significantly shortened	Urabe Y et al., 1996
	Whole liver biopsy	Significantly shortened	Aikata H et al., 2000
Liver cirrhosis	Whole liver biopsy	Significantly shortened	Kitada T et al., 1995
	Whole liver biopsy	Significantly shortened	Urabe Y et al., 1996
	Whole liver biopsy	Significantly shortened	Miura N et al., 1997
	Hepatocytes	Significantly shortened	Wiemann SU et al., 2002
	Whole liver biopsy	Significantly shortened	Oh BK et al., 2003
	Whole liver biopsy	Significantly shortened	Aikata H et al., 2001
Chronic liver damage	Whole liver biopsy	Significantly shortened	Wiemann SU et al., 2005
Nonalcoholic fatty liver disease	Whole liver biopsy	Significantly shortened	Nakajima T et al., 2006
Chronic obstructive pulmonary disease (COPD)	Alveolar epithelial and endothelial cells	Significantly shortened	Tsuji T et al., 2006
	Lymphocytes	Significantly shortened	Morlá M et al., 2006
Idiopathic pulmonary fibrosis (IPF)	Leukocyte	Significantly shortened	Tsakiri KD et al., 2007
Facioscapulohumeral muscular dystrophy	Myoblasts	Significantly shortened	Vilquin JT et al., 2005
Depression	PB	Significantly shortened	Simon NM et al., 2006
Cognitive decline, dementia	PBMCs	Significantly shortened	Martin-Ruiz C et al., 2006
Vascular dementia	Fibroblasts and PBMCs	Significantly shortened	von Zglinicki T et al., 2000
Atopic dermatitis and psoriasis	PB leucocytes	Significantly shortened	Wu K et al., 2000
Autosomal dominant dyskeratosis congenita (ADDC)	Granulocyte, CD45RA-negative memory T-cells and CD57-positive NK/NKT	No difference	Alter BP et al., 2007

Table 2 (continued)

Disease	Cell type	Telomere length change	Publication
Coronary disease	Leukocytes	Without correlation	Kurz DJ et al., 2006
Cardiovascular causes, infectious diseases	PB	No difference	Martin-Ruiz CM et al., 2005
Chronic ulcerative colitis	PB lymphocytes	No difference	Getliffe KM et al., 2005
Type 2 diabetes	Lymphocyte	No difference	Sampson MJ et al., 2006
Duchenne muscular dystrophy (DMD)	Dystrophic tissue	Slight shortening	Oexle K et al., 1997, 2001
HIV type 1 infection	CD4(+) T cell	No difference	Wolthers KC et al., 1997
	CD45RA+ naive and CD45RO+ memory CD4+ T cells	No difference	Wolthers KC et al., 1999
	CD4+, CD8+, CD45RA+ or CD45RO+ T-cells	No difference	Tucker V et al., 2000

PBMCs Peripheral blood mononuclear cells, *PB* peripheral blood, *BM* bone marrow, *WBCs* white blood cells

poiesis and increased myeloproliferation – two characteristic phenotypes of human ageing [32]. Another important observation in this study was that the alterations in the environment in telomere dysfunctional mice impairs the engraftment of transplanted stem cells. This observation could be highly relevant for new therapeutic approaches in regenerative medicine aiming to use stem cell transplantation for the treatment of ageing associated diseases.

Genetic evidence for a role of telomere shortening in human ageing

In addition to the association of telomere shortening with human disease (see above), mutations in critical components of the telomerase enzyme (Tert and Terc) have been associated with human disease. Terc mutations have been identified in patients with the autosomal dominant form of dyskeratosis congenita (DKC) – a disease syndrome leading to death from bone marrow failure at young age [34]. Another form of the disease is caused by mutation in dyskerin – a gene required for snoRNA processing [35]. It was shown that dyskerin also processes the RNA component of telomerase, and DKC patients with dyskerin mutations show reduced levels of Terc expression [35]. In addition, mutations in the catalytic subunit of telomerase (Tert) have been identified in different forms of anemia [36] and Tert mutations are also linked to the development of idiopathic pulmonary fibrosis in ca. 10% of the cases [37, 38]. Together, these studies show that mutations in a single copy of human telomerase genes have severe effects on maintenance of different organ systems and lead to a reduction in lifespan. These findings show that human telomeres are limited and changes in telomerase gene expression can affect organismal fitness and lifespan. It is tempting to speculate that more subtle differences in telomerase expression (for example promoter polymorphisms, epigenetic alterations) could have an impact on human health later in life.

Telomere shortening and cancer

The incidence of cancer sharply increases during ageing. Cancer can thus be seen as an ageing associated disease. Telomere shortening appears to have a dual role in cancer formation. Originally, it was proposed that telomere shortening limits the lifespan of human cells thus acting as a tumor suppressor mechanism [8]. According to this hypothesis cancer cells need to stabilize telomeres in order to gain immortal proliferative capacity – one of the hallmarks of cancer cells. In agreement with this hypothesis, more than 90% of human cancers show a strong reactivation of telomerase [28]. In contrast, the vast majority of human cancers exhibit very short telomeres, much shorter than the surrounding non-transformed tissue [28].

Studies in telomerase deficient mice have provided a plausible explanation for this apparent paradox. These studies have shown that telomere dysfunction increases the rate of cancer initiation by inducing chromosomal instability (CIN). Telomere dysfunction induces DNA damage response (see above) including the activation of DNA damage repair. The most frequent pathway of DNA repair in mammalian cells is the non-homologues end-joining (NHEJ) pathway. The activation of NHEJ also leads to formation of chromosomal fusions in response to telomere dysfunction [39]. In addition, it was shown that NHEJ-independent mechanisms can induce chromosomal fusion in response to telomere shortening [40]. When cells with fused chromosomes enter the cell cycle, the fusion has to be disrupted

Table 3 Telomerase mutation and disease

Disease	Mutation	Publication
Adult-onset pulmonary fibrosis	TERT	Tsakiri KD et al., 2007
	TERC	Tsakiri KD et al., 2007
Idiopathic pulmonary fibrosis	TERT	Armanios MY et al., 2007
	TERC	Armanios MY et al., 2007
Bone marrow failure	TERT	Vulliamy TJ et al., 2005
Aplastic anaemia	TERC	Vulliamy et al., 2002
	TERC	Xin ZT et al., 2007
	TERT	Liang J et al., 2006
	TERT	Yamaguchi H et al., 2005
	TERT	Xin ZT et al., 2007
Autosomal dominant dyskeratosis congenita (ADDC)	TERC	Vulliamy TJ et al., 2006
	TERC	Armanios M et al., 2006
	TERC	Xin ZT et al., 2007
	TERC	Vulliamy T et al., 2004
	TERC	Goldman F et al., 2005
	TERC	Chen JL et al., 2004
	TERC	Wong JM et al., 2006
	TERC	Walne AJ et al., 2007
	TERC	Wong JM et al., 2006
	TERC	Ly H et al., 2005
	TERC	Cerone MA et al., 2005
	TERC	Knudson M et al., 2005
	TERC	Theimer CA et al., 2003
	TERC	Lin JH et al., 2002
	TERC	Vulliamy T et al., 2002
	TERC	Vulliamy T et al., 2001
	TERC	Mitchell JR et al., 1999
	TERT	Xin ZT et al., 2007
	TERT	Armanios M et al., 2005
MDS	TERC	Ly H et al., 2005
	TERC	Ohyashiki K et al., 2005
Cri-du-chat syndrome	TERT	Zhang et al., 2003
Coronary artery disease	TERT	Matsubara et al., 2006

during anaphase – the stage when chromosomes move to opposite poles of the spindle during mitosis. This process leads to gains and losses of chromosomal material in the daughter cells. In addition, new breakpoints are generated that will induce another round of fusion and breakage in the next round of cell division. These "fusion-bridge-breakage cycles" can contribute to the accumulation of CIN in ageing human cells and thus to the accumulation of genetic alteration leading to cellular transformation. Studies in mTerc–/– mice have shown that loss of checkpoint genes (specifically loss of p53) cooperates with telomere dysfunction to induce CIN and cancer initiation [41].

In contrast to the increase in chromosomal instability and tumour initiation, telomere shortening inhibits tumour progression and the formation of macroscopic tumours in mTerc–/– mice [42]. Impaired tumour progression in telomere dysfunctional

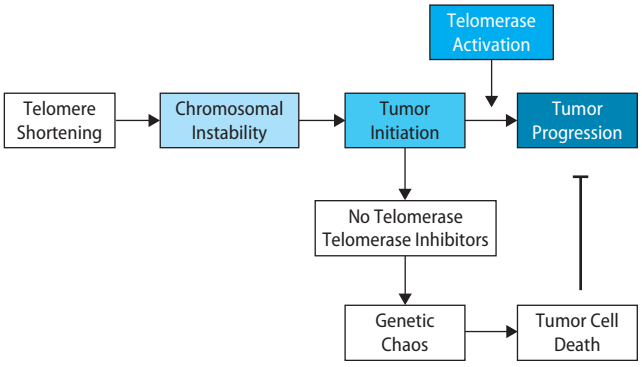

Fig. 1 Telomere shortening has a dual role in cancer formation. Telomeres shorten during ageing. In aged tissue a growing number of cells have short, dysfunctional telomeres. As a consequence chromosomal instability (CIN) increases, especially, when checkpoints genes are abrogated, such as p53. This leads to an increase in tumour initiation during ageing. However, tumour cell proliferation induces further telomere shortening and genetic chaos inhibiting tumour cell survival and tumour progression. At this stage, the activation of telomerase in tumour cells is required for tumour progression. Therefore, telomerase inhibitors represent a promising approach to treat telomerase-positive human cancer

mice correlated with an accumulation of high levels of CIN and DNA damage in tumour cells, as well as with impaired tumor cell proliferation and increased rates of tumour cell apoptosis [42]. Together these studies indicate that telomere shortening has a dual role in caner. On the one hand it can increase the initiation of tumours by inducing chromosomal instability and genetic alterations that lead to cellular transformation. On the other hand, tumour cells need to stabilize telomere shortening to avoid an accumulation of too high levels of instability that would ultimately kill the cancer cell (Fig. 1). This hypothesis appears to be in line with the observation that most human cancers have critically short telomeres but at the same time show a reactivation of telomerase (see above).

Conclusion

There is increasing evidence that telomere dysfunction has a causative role in human ageing, diseases, and cancer. Understanding the pathways and checkpoints that limit cellular function in response to telomere dysfunction could point to molecular targets for future therapies aiming to improve organ maintenance, regeneration and health in the growing population of the elderly. Moreover, these checkpoints are strongly connected to cancer formation and could thus be targets for future anti-cancer therapies. Telomere dysfunction and the activation of downstream pathways could also be biomarkers for human ageing and cancer risk. An investigation

of the consequences of telomere dysfunction on the stem cell level appears to be of utmost importance given that molecular alteration in the ageing stem cell likely impacts on ageing, diseases, and cancer formation.

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