HHS Public Access

Author manuscript

Trends Mol Med. Author manuscript; available in PMC 2021 April 01.

Published in final edited form as:

Trends Mol Med. 2020 April ; 26(4): 422–433. doi:10.1016/j.molmed.2020.01.010.

Noncoding RNAs controlling telomere homeostasis in senescence and aging

Martina Rossi, **Myriam Gorospe***

Laboratory of Genetics and Genomics, National Institute on Aging Intramural Research Program, National Institutes of Health, Baltimore, Maryland 21224, USA

Abstract

Aging is a universal and time-dependent biological decline associated with a progressive deterioration of cells, tissues, and organs. Age-related decay can eventually lead to pathologies such as cardiovascular and neurodegenerative diseases, cancer, and diabetes. A prominent molecular process underlying aging is the progressive shortening of telomeres, the structures that protect the ends of chromosomes, culminating in cellular senescence. Noncoding (nc)RNAs are emerging as major regulators of telomere length homeostasis. In this review, we describe the impact of ncRNAs on telomere function and discuss their implications in senescence and age-related diseases. We discuss emerging therapeutic strategies targeting telomere-regulatory ncRNAs in aging pathologies.

Keywords

Long noncoding RNAs; *TERC*; TERT; microRNA; telomerase activity; genomic instability

Noncoding RNAs In Telomere Dynamics

Only ~2% of the mammalian genome is transcribed into protein-coding RNAs, and the remaining ~98% was long considered to be inactive material (“junk DNA”). However, with the arrival of new technologies and analytical tools, noncoding (nc)RNAs have emerged as rich, abundant, and potent regulators of gene expression programs in cellular processes like proliferation, apoptosis, differentiation, and senescence [1–3]. NcRNAs broadly include all RNAs from which there is no evidence of protein translation. The functions of regulatory RNAs become particularly relevant in times of cellular stress when transcription and translation are globally suppressed or restricted to certain mRNA subsets. In addition, they offer therapeutic opportunities that cannot be achieved by targeting coding RNAs (mRNAs).

*Correspondence: myriam-gorospe@nih.gov (M.Gorospe).

RESOURCES

The large and heterogeneous class of ncRNAs can be divided into two main groups according to their size: long ncRNAs, typically spanning several hundred nucleotides, and short RNAs, generally in the range of 20–30 nucleotides [4]. Among a broad range of mechanisms of gene regulation, some ncRNAs have been shown to regulate transcription by interacting with chromatin-modifying complexes or by interfering with or enhancing the transcriptional machinery [5–7]. Other ncRNAs modulate key post-transcriptional steps by influencing mRNA processing, transport, storage, degradation, and translation. Many ncRNAs have been annotated thus far, but our understanding of their roles in gene regulation is in its infancy.

Telomeres are protective structures at the end of linear chromosomes comprising repeats of the tandem sequence TTAGGG and the shelterin complex (TRF1, TRF2, POT1, RAP1, TIN2, TPP1). Telomeres prevent DNA damage and end-to-end fusions of chromosomes (Figure 1) and shorten progressively with each cell division due to a defect in the DNA replicative mechanism known as the ‘end-replication problem’. Upon removal of the last RNA primer at the 3′ end of the lagging strand, the newly synthesized strand will be a few nucleotides shorter, resulting in telomere shortening. The progressive loss of telomeres causes exposure of the DNA ends, eventually triggering a DNA Damage Response (DDR) that culminates with chromosomal instability and the formation of aberrant chromosome end-to-end fusions [8, 9].

Noncoding (nc)RNAs are emerging as major regulators of telomere length homeostasis. Here, we describe the impact of ncRNAs on the telomere biology and in particular their possible implications in senescence and age-related pathologies associated with telomere dysfunctions (Figure 1). Specifically, our discussion focuses on two types of regulatory RNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs).

Telomeres in Senescence and Age-related Diseases

The enzyme telomerase counteracts telomere shortening by replenishing telomeric repeats. In this manner, telomerase protects the genetic information from being truncated progressively during cell division [8–10]. However, most somatic cells have no detectable telomerase and undergo telomere shortening, which eventually leads to loss of telomere capping and triggers DDR. In cells with functional checkpoints, the DDR leads to increased levels of the transcription factor TP53 (p53), which is involved in DNA repair, cell cycle arrest, and apoptosis. TP53 transcriptionally induces the production of p21 (CDKN1A), a cyclin-dependent kinase (CDK) inhibitor that can halt cell division [9, 10]. Additionally, telomere dysfunction may also increase the levels of the CDK inhibitor p16 (CDKN2A) which further suppresses cell growth. Both p21 and p16 enable the function of the retinoblastoma protein (RB) and reinforce the cell cycle arrest [11, 12]. Therefore, in cells with functional cell cycle regulators, critical telomere shortening is associated with reduced proliferative capacity. Cells then enter a state of indefinite growth arrest and become senescent, although they remain metabolically active.

By contrast, impairment of checkpoints and tumor suppressor pathways causes cells to continue dividing and escape from replicative senescence. Fully unprotected chromosome

ends can form end-to-end fusions leading to genomic instability and a state of “crisis” associated with massive cell death. Given that the reorganization of the genome allows the generation of pre-neoplastic cells [8–10], most tumor cells have devised mechanisms to maintain telomeres of a certain minimal size by reactivating the telomerase reverse transcriptase, thus expanding their replicative potential. The enzyme telomerase is composed of two core components, the telomerase reverse transcriptase (TERT) and the telomerase RNA (*TERC*, *TE*lomerase *R*NA *C*omponent, also known as *hTR*), which serves as the template for TERT in the addition of telomeric repeats. The active telomerase complex requires other ancillary components, including the binding partners dyskerin (DKC1), NOP10, NHP2, GAR1, and Telomerase Cajal body protein 1 (TCAB1) [13]. In a small percentage of telomerase-negative immortalized cells and cancer cells, alternative lengthening of telomere (ALT) is achieved through homologous recombination-dependent synthesis of new telomeric DNA [9].

Senescent cells have been detected in multiple age-related diseases and are associated with loss of tissue function during the aging process. Besides cell cycle arrest, senescent cells undergo changes in morphology, gene expression patterns, and metabolic programs. Importantly, senescent cells display a distinct trait known as the **senescence-associated secretory phenotype (SASP),** whereby they secrete high levels of pro-inflammatory cytokines capable of altering the microenvironment, triggering immune surveillance, enhancing angiogenesis, and remodeling the extracellular matrix, with a range of beneficial and deleterious effects on different organs [14–17]. The causes, consequences, and phenotypes of cell senescence have been reviewed in detail [17–20].

Cellular senescence may also occur independently of telomere shortening; for example, DNA damage, oxidative injury, and oncogenic signaling are able to induce cellular senescence. Stress- and oncogene-induced senescence may also incur acute damage at the telomeres and trigger DDR signaling, in turn suppressing cell proliferation [21–23]. According to this evidence, telomeres not only determine the rounds of division of a cell, but also act as sensors of intrinsic and extrinsic stresses to suppress the proliferation of cells that have accumulated significant genomic damage [10].

Several lines of evidence support the notion that short telomeres are associated with premature aging and age-related diseases [10, 24]. First, an inverse association exists between chronological age and telomere length, even though telomere length is highly heterogeneous among different tissues and different individuals of the same age [24, 25]. We note that it is unclear whether telomere length is a useful biomarker of aging and cellular senescence, as discussed elsewhere [24–28]. Second, accelerated aging conditions known as Short Telomere Syndromes (STSs) are caused by gene mutations resulting in premature telomere attrition and multisystemic diseases such as Dyskeratosis Congenita, Werner’s Syndrome, Hutchinson-Gilford Syndrome and Ataxia-telangiectasia. Patients with these syndromes often show phenotypes of accelerated aging such as diabetes, myocardial infarction, and cognitive decline [29, 30]. Third, telomere shortening has been associated with common age-related diseases including cardiovascular disease, diabetes, neurodegeneration, chronic obstructive pulmonary disease (COPD), and skin disorders [25, 31]. Fourth, many age-related diseases are exacerbated by immune cell senescence. For

these reasons, the study of telomere attrition in immune cells like leukocytes is relevant for the etiology of these conditions and may serve as surrogate of telomere length in other tissues [29]. Alterations in leukocyte telomere length (LTL) is associated with increased risk of many cancer types [25, 29, 31, 32], supporting the notion that premalignant cells have lost telomere length homeostasis, prior to the activation of telomerase-mediated telomere maintenance. In sum, understanding the molecular processes underlying telomere dysfunction could help identify possible therapeutic targets in age-related diseases.

microRNAs Regulating Telomere Dynamics in Senescence and Aging

MicroRNAs (miRNAs) are a major class of short ncRNAs that regulate gene expression programs post-transcriptionally. They generally function by binding to specific mRNAs through complementary sequences and inducing degradation and/or blocking the translation of the target mRNA [33]. Many microRNAs control the expression of factors that influence telomere length dynamics (Figure 1). In this section, we focus on the senescence-associated microRNAs (SA-miRNAs) that have been shown to play a role in senescence or aging by influencing telomere maintenance (Table 1).

miR-23a.

MicroRNA miR-23a was initially found to promote cellular senescence in human fibroblasts by inhibiting the expression of TRF2, a key component of the shelterin complex (Figure 1) [34]. In a later study, Satoh *et al.* [35] showed that high levels of miR-23a not only induced telomere shortening, but were also associated with poor clinical outcomes in patients with coronary artery disease (CAD), an age-related condition. Furthermore, the high levels of miR-23a in the CAD group were inversely correlated to the levels of TRF2 protein [35].

miR-34a.

This microRNA was recognized as a major regulator of the levels of surtuins, a family of NAD-dependent deacetylases that perform a variety of functions in several cellular compartments, including histone modification and control of metabolic proteins. Among them, SIRT1, SIRT6 and SIRT7, may localize at chromosome ends and maintain telomere length. Previous studies have shown that the deacetylase activity of sirtuins promotes telomere integrity by maintaining chromatin condensation and by promoting the interaction between histones and telomere regulatory factors, like components of the DNA repair machinery. Moreover, sirtuins suppress the DNA damage response at the telomere by inactivating TP53 [36, 37]. MiR-34 was recognized as a major repressor of the levels of SIRT1, SIRT6 and SIRT7 [38–40].

In cardiomyocytes, miR-34a modulated telomere maintenance by reducing the abundance of protein Phosphatase 1 NUclear Targeting Subunit (PNUTS, also known as PPP1R10), which directs protein phosphatase 1 (PP1) to the nucleus. PNUTS was previously found to inhibit the DDR and to interact with TRF2, further preventing telomere shortening [41, 42]. Furthermore, miR-34a induced senescence of human hepatocellular carcinoma (HCC) cells by modulating telomerase activity. In HCC tumors, the levels of miR-34a correlated inversely with telomere length and telomerase activity, while increasing miR-34a ectopically

in HCC cells triggered senescence and affected cell viability. The actions by miR-34a on telomere maintenance were attributed to the miR-34a-mediated suppression of the Forkhead Box M1 (FOXM1) and MYC pathway, required for *hTERT* transcription and telomerase activity [43].

miR-195.

Compared to young mesenchymal stems cells (YMSCs), in which telomerase is highly abundant and active, old mesenchymal stem cells (OMSCs) express high levels of miR-195. The 3'UTR of *TERT* mRNA was identified as a putative target of miR-195 and depletion of miR-195 rescued hTERT expression and cell proliferation in OMSCs. Importantly, injection of OMSCs with depleted miR-195 significantly improved the regeneration of mouse heart after infarction [44]. A further study showed that miR-195 promoted senescence in skeletal muscle cells by lowering the production of SIRT1 [45]

miR-126.

A rise in miR-126 abundance delayed the senescence of human glomerular mesangial cells (HGMCs) induced by high glucose. While high glucose led to shortened telomeres, miR-126 upregulation was associated with extended telomeres and decreased expression of DNA damage response and /or senescence markers TP53 and p21 [46]. Another study found that deletion of miR-126a promoted hepatic aging in mice and induced age-associated telomere shortening [47].

miR-155.

MiR-155 was found elevated in breast cancer specimens, together with reduced levels of TRF1, a protein component of the shelterin complex (Figure 1). Accordingly, in breast cancer cells it was shown that miR-155 interacts with the 3'UTR of the *TRF1* mRNA and represses the translation of the TRF1 protein. The increased levels of miR-155 were associated with telomere and genomic instability, and with poor clinical outcome in estrogen receptor-positive breast cancer [48]. Notably, miR-155 levels are often reduced in senescent cells [49].

Other microRNAs.

Additional microRNAs (miR-570, miR-217, miR-138, miR-22, miR-181a, miR-449, mir-26, mir-145a, miR-766) were reported to suppress the expression of sirtuin family members SIRT1 and SIRT6 [39, 40, 50–56], while other microRNAs modulate the expression of telomere components TERT (miR-128, miR-296–5p, and miR-512–5p) [57–59] and DKC1 (miR-150) [60]. Interestingly, some microRNAs were themselves regulated by hTERT levels in senescent cells, although the mechanisms are not well understood (miR-143, miR-145, miR-146 [49]). Finally, the expression levels of a subset of microRNAs (let-7d-5p, let-7e-5p, miR-125a-3p, miR-125a-5p, miR-125b-5p, miR-23a-3p, miR-34a-5p, miR-181a-5p) increased upon shortening of telomeres in senescent fibroblasts, and they in turn suppressed the production of various cell cycle regulatory transcripts [61].

Taken together, microRNAs can promote or suppress telomere function by modulating the response of telomeres to DNA damage, by suppressing the levels of proteins that protect telomere ends, and by influencing the levels of telomerase components (Figure 1).

lncRNAs Impacting Telomere Maintenance in Senescence and Aging

Several lncRNAs have been associated with the integration of the DDR, the protection of telomere ends and the maintenance of telomere length (Figure 1).

TERC (hTR)

The telomerase core component *hTR* (human telomerase RNA) is encoded by the *TERC* gene, located on the chromosomal region 3q26. *TERC* serves as a template for TERT by providing a sequence (AAUCCC) for the insertion of the repetitive G-rich DNA sequence 5′-TTAGGG-3′ to the ends of chromosomes [62]. *TERC* is involved in the localization and assembly of the telomerase holoenzyme, and modifications of specific *TERC* residues were shown to compromise the catalytic activity of the telomerase [63]. *TERC* contains three major structural and functional domains: a core domain featuring the template sequence, a Stem Terminus Element (STE, featuring conserved regions CR4/CR5 domain) essential for the telomerase catalytic activity, and a 3' terminal domain (known as H/ACA small Cajal body-specific RNA) that contains specific signals for *TERC* processing and localization [63]. In 1997, the development of a telomerase-deficient ($TERC^{-/-}$) mouse provided a model for the study of telomere function in cancer, cardiovascular disease, and other age-related pathologies. The phenotype of late generations of $TERC^{-/-}$ mice included chromosome end fusions, decreased lifespan, and typical features of aging like atrophy and reduced angiogenesis [64, 65].

Genetic variations of the human *TERC* gene, as well as other telomere maintenance genes, can alter the stability and catalytic activity of the telomerase complex. In line with this notion, *TERC* mutations have been linked to telomere biology disorders [66] and to shorter human lifespan [67]. For example, mutations in *TERC* cause autosomal forms of dyskeratosis congenita (DC), characterized by shorter telomeres, cancer disposition, bone marrow failure, and premature aging. Although disease-associated *TERC* mutations are distributed throughout the RNA, the majority of them map to sequences essential for telomerase catalysis, a specific TERT-binding site, and the template region, causing reduced catalytic activity and aberrant addition of telomeric repeats [68].

Shorter telomeres were associated with the pathogenesis and development of age-associated cognitive decline and Alzheimer's disease (AD). In particular, *TERC* genetic variations in AD patients were compared with the frequencies observed in control patients. Specific combinations of single-nucleotide polymorphisms (SNPs) of the *TERC* gene were found to affect the age at AD onset [69].

In addition, specific *TERC* mutations were found to be associated with a higher risk of Idiopathic Pulmonary Fibrosis (IPF), an aging-related syndrome characterized by interstitial lung scarring, as well as premature hair greying, bone marrow failure, and liver cirrhosis.

IPF is thought to arise from the impaired regeneration of damaged alveolar epithelial cells in the presence of diminished telomerase function [70–73].

A recent study identified an alternative regulatory role for *TERC* on senescence and aging independent of its function in the telomerase complex [74]. *TERC* was found to be imported into mitochondria and processed to a shorter form, *TERC-53*, that was exported back to the cytosol. *TERC-53* accumulated in the cytosol upon cellular stress and may serve as an indicator of mitochondrial function [74, 75]. Interestingly, overexpression of *TERC-53* in human fibroblasts induced cellular senescence, and overexpression of *mTerc-53* in mice accelerated cognitive decline; these interventions did not affect telomerase activity, but rather caused stem cell exhaustion and proliferative decline [74].

Recent evidence indicates that the mechanisms adopted by tumor cells to activate telomerase and avoid telomere shortening include amplification of the *TERC* gene, besides the well-known derepression of the *TERT* gene. In this manner, telomerase activation contributes to the evasion of cancer cells beyond the normal limits of proliferation [76, 77].

TERRA

Despite being heterochromatic, telomeric regions are transcribed into lncRNAs collectively known as *TERRA* (TElomeric Repeat-containing RNA) that play crucial roles in telomere protection and maintenance. *TERRA* transcription starts from the subtelomeric region and then proceeds into telomere repeats. In mammals, *TERRA* molecules have different lengths, ranging between 100 nt and 9 kb [78, 79].

TERRA transcripts can protect chromosome ends by promoting telomere maintenance [80, 81]. The G-rich 3′ end of *TERRA* folds into multimeric G-quadruplex structures composed of four G rings that interact with several capping molecules located at the telomere ends, such as the shelterin components TRF1 and TRF2 [82–85]. *TERRA* RNAs also interact with proteins that contribute to maintaining the heterochromatic state, including the Polycomb Repressive Complex 2 (PRC2), which has histone methyltransferase activity [86, 87]. The interaction between *TERRA* transcripts and PRC2 enhances the deposition of heterochromatin marks like H3K9me3, H3K27me3, H4K20me3 and HP1 at chromosome ends [86, 87]. At extratelomeric sites, the interaction between *TERRA* and epigenetic factors can regulate chromatin remodeling and transcription [88]. Finally, *TERRA* transcripts form RNA-DNA hybrid structures named R-loops, which influence telomere heterochromatin assembly, replication, and homologous recombination among telomeres, and can thereby impact the onset of senescence [88–91].

TERRA RNAs have been shown to modulate the activity of the telomerase. As *TERRA* transcripts contain tandem G-rich sequences (UUAGGG) with strong affinity for *TERC* [92], they may act as direct inhibitors of telomerase activity [93]. Moreover, *TERRA* transcripts may bind TERT directly and modulate telomerase activity independently of *TERC* [93]; accordingly, *TERRA*-mimicking oligonucleotides inhibited telomerase activity [94], while *TERRA* depletion increased it [88]. However, the role of *TERRA* on telomerase activity remains controversial, with evidence that *TERRA* transcripts were capable of

recruiting telomerase to critically short telomeres to promote telomere elongation [95], and that high levels of *TERRA* did not affect telomere elongation in human cancer cells [96].

The molecular functions of *TERRA* on telomere maintenance in senescence are also unclear. On one hand *TERRA* may protect telomere ends, but on the other it can counteract telomere elongation. Overexpression of *TERRA* in telomerase-negative cells delayed the onset of senescence [91], and masked 3' overhangs of uncapped telomeres during DNA damage-induced senescence, thus protecting telomere ends [97]; however, in other studies high levels of *TERRA* triggered premature senescence by suppressing telomere elongation and DNA replication [98, 99].

The role of *TERRA* in aging is also poorly understood. A recent review [100] suggested that an interplay between *TERRA* and *TERC* regulates telomerase activity and the survival rate of neural stem cells during aging. Therefore, shifts in abundance or activity of these molecules might be involved in age-associated changes [100]. Other studies revealed that *TERRA* levels were high in blood mononuclear cells of patients with IPF, while *TERRA* silencing improved telomere function [70, 101].

The levels of *TERRA* transcripts vary widely and differ among stages of cancer progression [102]. *TERRA* transcripts were reduced in telomerase-positive cancer cells, where the subtelomeric translational regions are highly methylated [103], but they were elevated in ALT-positive cancers [95, 102, 103]. These differences may be related to the fact that telomeric chromatin is less compact in ALT-positive cells than in telomerase-positive cells [80, 103].

In summary, even though *TERRA* functions have not yet been fully elucidated, the tight regulation of *TERRA* levels production appears to be necessary for maintaining telomere homeostasis, with consequences on cancer, senescence, and aging.

GUARDIN

The lncRNA *GUARDIN*, a transcriptional target of TP53, was shown to be important for maintaining genomic integrity both in unstimulated cells and in cells responding to DNA damage. *GUARDIN* protected telomere ends from damage and prevented chromosome end-to-end fusion in large part by sequestering miR-23a, and thereby ensuring the production of the shelterin component TRF2. In addition, *GUARDIN* promoted DNA repair by acting as a scaffold to facilitate the heterodimerization of breast cancer type 1 susceptibility protein (BRCA1) and the BRCA1-associated RING domain 1 (BARD1) protein, which stabilizes BRCA1. *GUARDIN* silencing not only induced apoptosis and cellular senescence, but it also reduced the growth of cancer xenografts in mice and sensitized cancer cells to genotoxic drugs [104].

Therapies Directed at Telomere-Regulatory ncRNAs: Progress and Prospects

The past decade has seen an escalation in the development of therapeutic approaches exploiting noncoding RNAs, mainly microRNAs and lncRNAs [105, 106]. Some

interventions directed at microRNAs may be advantageous because a microRNA may jointly suppress multiple proteins implicated in a given phenotype, or it may suppress proteins selectively expressed in a specific cell type. Current therapeutic strategies to overexpress a specific miRNA involves the delivery of synthetic oligoribonucleotides that mimic the native miRNA, often bearing modifications and complexed molecules for stability and efficacy. Conversely, suppression of microRNA actions often relies on the delivery of antisense oligonucleotides that neutralize the endogenous microRNA and block its activity [107, 108].

Similarly, lncRNAs represent a class of attractive drug targets, given their dysregulation in disease and their tissue-specific expression. For example, many lncRNAs interact with the repressive PRC2 machinery and thus may have a broad impact on gene expression patterns. In this regard, different oligonucleotides have been developed to induce cleavage of a specific lncRNA or to compete with PRC2 for the association with a target lncRNA [106, 109].

Major challenges in RNA-directed oligonucleotide therapeutics are the toxicity at the therapeutic dose, the binding to unintended targets, the stability of the oligonucleotide, and the delivery to the intended tissue. Nanotechnology-based delivery systems have improved greatly the stability and target tissue specificity of therapeutic RNAs, while chemical modifications have improved the pharmacokinetic properties of the oligonucleotides and have lessened their toxicity and off-target actions [105, 106].

Several RNA-based therapeutics have been developed to treat age-related pathologies like cardiovascular disease, diabetes, neurodegeneration, and cancer [105]. Interestingly, some of the ncRNAs targeted therapeutically influence telomere maintenance. For example, in preclinical studies, the miR-34a mimic MRX34 reduced the growth of non-small cell lung cancer in mice and prolonged survival [110]. However, the phase 1 clinical trial of MRX34 was terminated due to adverse immune complications in some patients (NCT01829971)[I] . Anti-miR-155 molecules are being investigated for the treatment of cutaneous T cell lymphoma (CTCL) and have successfully passed through phase 1 of clinical trials (NCT03837457) [II] . MiR-155 inhibitors are currently investigated also for the treatment of neuroinflammatory and neurodegenerative diseases [111].

LncRNAs linked to telomere function are also being evaluated as therapeutic targets. Given earlier evidence that inhibiting telomerase could be an effective therapeutic approach in various cancers, oligonucleotides or small molecules specifically directed at *TERRA* and *TERC* are being developed with the hope of modulating telomerase activity and telomere length. For example, the oligonucleotide Imetelstat binds with high affinity the template region of *TERC*, directly inhibiting telomerase activity. Phase 1 trials were successfully completed and clinical phase 2 trials are underway (NCT02598661) [III] . Strategies directed at *TERC* inhibition include the design of hammerhead ribozymes, RNA molecules able to cleave the target RNA in a site-specific manner, resulting in a reduced telomerase activity [112, 113].

[I] https://clinicaltrials.gov/ct2/show/NCT01829971
[II] https://clinicaltrials.gov/ct2/show/NCT03837457 ; http://www.miragen.com/pipeline/
[III] https://clinicaltrials.gov/ct2/show/NCT02598661 ; https://www.geron.com/r-d/imetelstat/

TERRA RNAs may negatively regulate the telomerase activity and are often dysregulated in cancer and age-related diseases. Sinha *et al.* [102] recently reviewed several small molecules, like BRACO-19, which possess anti-cancer potential and are able to stabilize the telomeric DNA G-quadruplexes [114]. Given that ligand-induced telomeric G-quadruplex stabilization was shown to inhibit the telomerase activity, it will be interesting to investigate whether *TERRA* G-quadruplexes might be involved in the proposed mechanism and could be therapeutic targets.

In closing, the development of oligonucleotides, small molecules and other strategies targeting ncRNAs that modulate telomere function, together with the advancements in delivery systems, will further help in the development of new treatments against cancer and other age-related diseases (see Clinician's Corner).

Concluding Remarks

NcRNAs are major regulators of telomere dynamics, which are well-known for playing an essential role in cellular senescence, aging and cancer. We have described the broad, pleiotropic action of miRNAs in several pathways involved in telomere homeostasis. We have also reported the role of specific lncRNAs in chromosome ends protection and regulation of the telomerase activity. However, despite progresses made in RNA studies, several questions still need to be addressed (see Outstanding Questions). Besides chromosome ends protection and DNA damage sensing, telomeres play an important role in other biological processes. An area of telomere research which has been unexplored for long time is the chromosomal looping between telomeres and distant genic regions. This long-distance interaction was shown to regulate gene expression and was shown to modulate, among other transcripts, the expression of ncRNAs [115]. Whether ncRNAs might in turn regulate the process of telomere looping in long-distance interactions remains to be determined.

Most studies have focused on the role of telomeres in tumor suppressor mechanisms and cellular senescence. For this reason, an area of particular interest is the development of therapeutic strategies targeting telomeric ncRNAs in cancer and age-related diseases. Notably, some of these compounds have already passed the first phase of clinical trials. Future research will probably identify tissue-specific ncRNAs with a role in telomere dynamics, allowing a more precise intervention in the context of therapeutic strategies.

ACKNOWLEDGEMENTS

This work was supported by the NIA IRP, NIH. We appreciate the advice of Dr. Yie Liu (NIA).

REFERENCES

1. Birney E, et al., Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature, 2007 447(7146): p. 799–816. [PubMed: 17571346]
2. Taft RJ, Pheasant M, and Mattick JS, The relationship between non-protein-coding DNA and eukaryotic complexity. Bioessays, 2007 29(3): p. 288–99. [PubMed: 17295292]
3. de Andres-Pablo A, Morillon A, and Wery M, LncRNAs, lost in translation or licence to regulate? Curr Genet, 2017 63(1): p. 29–33. [PubMed: 27230909]

4. Clancy S, RNA Functions, in Nature Education. 2008
5. Clemson CM, et al., An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell, 2009 33(6): p. 717–26. [PubMed: 19217333]
6. Prasanth KV, et al., Regulating gene expression through RNA nuclear retention. Cell, 2005 123(2): p. 249–63. [PubMed: 16239143]
7. Sunwoo H, et al., MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. Genome Res, 2009 19(3): p. 347–59. [PubMed: 19106332]
8. Shay JW, Telomeres and aging. Curr Opin Cell Biol, 2018 52: p. 1–7. [PubMed: 29253739]
9. Bernal A and Tusell L, Telomeres: Implications for Cancer Development. Int J Mol Sci, 2018 19(1).
10. Victorelli S and Passos JF, Telomeres and Cell Senescence - Size Matters Not. EBioMedicine, 2017 21: p. 14–20. [PubMed: 28347656]
11. Jacobs JJ and de Lange T, Significant role for p16INK4a in p53-independent telomere-directed senescence. Curr Biol, 2004 14(24): p. 2302–8. [PubMed: 15620660]
12. Zhang X, et al., Loss of p16(Ink4a) function rescues cellular senescence induced by telomere dysfunction. Int J Mol Sci, 2012 13(5): p. 5866–77. [PubMed: 22754337]
13. Venteicher AS, et al., A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. Science, 2009 323(5914): p. 644–8. [PubMed: 19179534]
14. Acosta JC, et al., A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol, 2013 15(8): p. 978–90. [PubMed: 23770676]
15. Coppé JP, et al., Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol, 2008 6(12): p. 2853–68. [PubMed: 19053174]
16. Demaria M, et al., Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. Cancer Discov, 2017 7(2): p. 165–176. [PubMed: 27979832]
17. van Deursen JM, The role of senescent cells in ageing. Nature, 2014 509(7501): p. 439–46. [PubMed: 24848057]
18. Kuilman T, et al., The essence of senescence. Genes Dev, 2010 24(22): p. 2463–79. [PubMed: 21078816]
19. Munoz-Espin D and Serrano M, Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol, 2014 15(7): p. 482–96. [PubMed: 24954210]
20. Salama R, et al., Cellular senescence and its effector programs. Genes Dev, 2014 28(2): p. 99–114. [PubMed: 24449267]
21. Fumagalli M, et al., Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat Cell Biol, 2012 14(4): p. 355–65. [PubMed: 22426077]
22. Kaul Z, et al., Five dysfunctional telomeres predict onset of senescence in human cells. EMBO Rep, 2011 13(1): p. 52–9. [PubMed: 22157895]
23. Suram A, et al., Oncogene-induced telomere dysfunction enforces cellular senescence in human cancer precursor lesions. EMBO J, 2012 31(13): p. 2839–51. [PubMed: 22569128]
24. Bernadotte A, Mikhelson VM, and Spivak IM, Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. Aging (Albany NY), 2016 8(1): p. 3–11. [PubMed: 26805432]
25. Chilton W, O'Brien B, and Charchar F, Telomeres, Aging and Exercise: Guilty by Association? Int J Mol Sci, 2017 18(12).
26. Fasching CL, Telomere length measurement as a clinical biomarker of aging and disease. Crit Rev Clin Lab Sci, 2018 55(7): p. 443–465. [PubMed: 30265166]
27. Mather KA, et al., Is telomere length a biomarker of aging? A review. J Gerontol A Biol Sci Med Sci, 2011 66(2): p. 202–13. [PubMed: 21030466]
28. Sanders JL and Newman AB, Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? Epidemiol Rev, 2013 35: p. 112–31. [PubMed: 23302541]
29. Blackburn EH, Epel ES, and Lin J, Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science, 2015 350(6265): p. 1193–8. [PubMed: 26785477]

30. Opresko PL and Shay JW, Telomere-associated aging disorders. Ageing Res Rev, 2017 33: p. 52–66. [PubMed: 27215853]
31. Arsenis NC, et al., Physical activity and telomere length: Impact of aging and potential mechanisms of action. Oncotarget, 2017 8(27): p. 45008–45019. [PubMed: 28410238]
32. Shay JW, Role of Telomeres and Telomerase in Aging and Cancer. Cancer Discov, 2016 6(6): p. 584–93. [PubMed: 27029895]
33. Bartel DP, MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004 116(2): p. 281–97. [PubMed: 14744438]
34. Luo Z, et al., Mir-23a induces telomere dysfunction and cellular senescence by inhibiting TRF2 expression. Aging Cell, 2015 14(3): p. 391–9. [PubMed: 25753893]
35. Satoh M, et al., Expression of miR-23a induces telomere shortening and is associated with poor clinical outcomes in patients with coronary artery disease. Clin Sci (Lond), 2017 131(15): p. 2007–2017. [PubMed: 28646123]
36. Amano H and Sahin E, Telomeres and sirtuins: at the end we meet again. Mol Cell Oncol, 2019 6(5): p. e1632613. [PubMed: 31528700]
37. Lee SH, et al., Sirtuin signaling in cellular senescence and aging. BMB Rep, 2019 52(1): p. 24–34. [PubMed: 30526767]
38. Baker JR, et al., Oxidative stress dependent microRNA-34a activation via PI3Kalpha reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. Sci Rep, 2016 6: p. 35871. [PubMed: 27767101]
39. Barnes PJ, Baker J, and Donnelly LE, Cellular Senescence as a Mechanism and Target in Chronic Lung Diseases. Am J Respir Crit Care Med, 2019.
40. Amano H, et al., Telomere Dysfunction Induces Sirtuin Repression that Drives Telomere-Dependent Disease. Cell Metab, 2019 29(6): p. 1274–1290 e9. [PubMed: 30930169]
41. Boon RA, et al., MicroRNA-34a regulates cardiac ageing and function. Nature, 2013 495(7439): p. 107–10. [PubMed: 23426265]
42. Marques FZ, et al., Telomere dynamics during aging in polygenic left ventricular hypertrophy. Physiol Genomics, 2016 48(1): p. 42–9. [PubMed: 26508703]
43. Xu X, et al., miR-34a induces cellular senescence via modulation of telomerase activity in human hepatocellular carcinoma by targeting FoxM1/c-Myc pathway. Oncotarget, 2015 6(6): p. 3988–4004. [PubMed: 25686834]
44. Okada M, et al., Abrogation of Age-Induced MicroRNA-195 Rejuvenates the Senescent Mesenchymal Stem Cells by Reactivating Telomerase. Stem Cells, 2016 34(1): p. 148–59. [PubMed: 26390028]
45. Kondo H, et al., Blockade of senescence-associated microRNA-195 in aged skeletal muscle cells facilitates reprogramming to produce induced pluripotent stem cells. Aging Cell, 2016 15(1): p. 56–66. [PubMed: 26637971]
46. Cao DW, et al., Upregulation of MiR-126 Delays the Senescence of Human Glomerular Mesangial Cells Induced by High Glucose via Telomere-p53-p21-Rb Signaling Pathway. Curr Med Sci, 2018 38(5): p. 758–764. [PubMed: 30341510]
47. Yan Y, et al., Deletion of miR-126a Promotes Hepatic Aging and Inflammation in a Mouse Model of Cholestasis. Mol Ther Nucleic Acids, 2019 16: p. 494–504. [PubMed: 31051334]
48. Dinami R, et al., miR-155 drives telomere fragility in human breast cancer by targeting TRF1. Cancer Res, 2014 74(15): p. 4145–56. [PubMed: 24876105]
49. Bonifacio LN and Jarstfer MB, MiRNA profile associated with replicative senescence, extended cell culture, and ectopic telomerase expression in human foreskin fibroblasts. PLoS One, 2010 5(9).
50. Menghini R, et al., MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation, 2009 120(15): p. 1524–32. [PubMed: 19786632]
51. Rivetti di Val Cervo P, et al., p63-microRNA feedback in keratinocyte senescence. Proc Natl Acad Sci U S A, 2012 109(4): p. 1133–8. [PubMed: 22228303]
52. Xu D, et al., miR-22 represses cancer progression by inducing cellular senescence. J Cell Biol, 2011 193(2): p. 409–24. [PubMed: 21502362]

53. Jazbutyte V, et al., MicroRNA-22 increases senescence and activates cardiac fibroblasts in the aging heart. Age (Dordr), 2013 35(3): p. 747–62. [PubMed: 22538858]
54. Zhou Y, et al., Protection of CD4+ T cells from hepatitis C virus infection-associated senescence via DeltaNp63-miR-181a-Sirt1 pathway. J Leukoc Biol, 2016 100(5): p. 1201–1211. [PubMed: 27354409]
55. Bou Kheir T, et al., miR-449 inhibits cell proliferation and is down-regulated in gastric cancer. Mol Cancer, 2011 10: p. 29. [PubMed: 21418558]
56. Sharma A, et al., The role of SIRT6 protein in aging and reprogramming of human induced pluripotent stem cells. J Biol Chem, 2013 288(25): p. 18439–47. [PubMed: 23653361]
57. Guzman H, et al., miR-128 inhibits telomerase activity by targeting TERT mRNA. Oncotarget, 2018 9(17): p. 13244–13253. [PubMed: 29568354]
58. Dinami R, et al., Epigenetic silencing of miR-296 and miR-512 ensures hTERT dependent apoptosis protection and telomere maintenance in basal-type breast cancer cells. Oncotarget, 2017 8(56): p. 95674–95691. [PubMed: 29221158]
59. Li J, et al., miR-512–5p suppresses tumor growth by targeting hTERT in telomerase positive head and neck squamous cell carcinoma in vitro and in vivo. PLoS One, 2015 10(8): p. e0135265. [PubMed: 26258591]
60. Watanabe A, et al., The role of microRNA-150 as a tumor suppressor in malignant lymphoma. Leukemia, 2011 25(8): p. 1324–34. [PubMed: 21502955]
61. Markopoulos GS, et al., Senescence-associated microRNAs target cell cycle regulatory genes in normal human lung fibroblasts. Exp Gerontol, 2017 96: p. 110–122. [PubMed: 28658612]
62. Jafri MA, et al., Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med, 2016 8(1): p. 69. [PubMed: 27323951]
63. Zhang Q, Kim NK, and Feigon J, Architecture of human telomerase RNA. Proc Natl Acad Sci U S A, 2011 108(51): p. 20325–32. [PubMed: 21844345]
64. Blasco MA, et al., Telomere shortening and tumor formation by mouse cells lacking telomerase RNA, in Cell. 1997: United States p. 25–34.
65. Wong LS, et al., Telomere biology in cardiovascular disease: the TERC−/− mouse as a model for heart failure and ageing, in Cardiovasc Res. 2009: England p. 244–52.
66. Sarek G, et al., Molecular basis of telomere dysfunction in human genetic diseases. Nat Struct Mol Biol, 2015 22(11): p. 867–74. [PubMed: 26581521]
67. Scarabino D, et al., Analysis of the Association Between TERC and TERT Genetic Variation and Leukocyte Telomere Length and Human Lifespan-A Follow-Up Study. Genes (Basel), 2019 10(2).
68. Nelson ND and Bertuch AA, Dyskeratosis congenita as a disorder of telomere maintenance. Mutat Res, 2012 730(1–2): p. 43–51. [PubMed: 21745483]
69. Scarabino D, et al., Common variants of human TERT and TERC genes and susceptibility to sporadic Alzheimers disease. Exp Gerontol, 2017 88: p. 19–24. [PubMed: 28039025]
70. Arish N, Petukhov D, and Wallach-Dayan SB, The Role of Telomerase and Telomeres in Interstitial Lung Diseases: From Molecules to Clinical Implications. Int J Mol Sci, 2019 20(12).
71. Armanios MY, et al., Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med, 2007 356(13): p. 1317–26. [PubMed: 17392301]
72. Borie R, et al., Prevalence and characteristics of TERT and TERC mutations in suspected genetic pulmonary fibrosis. Eur Respir J, 2016 48(6): p. 1721–1731. [PubMed: 27836952]
73. Tsakiri KD, et al., Adult-onset pulmonary fibrosis caused by mutations in telomerase. Proc Natl Acad Sci U S A, 2007 104(18): p. 7552–7. [PubMed: 17460043]
74. Zheng Q, et al., Mitochondrion-processed TERC regulates senescence without affecting telomerase activities. Protein Cell, 2019.
75. Cheng Y, et al., Mitochondrial Trafficking and Processing of Telomerase RNA TERC. Cell Rep, 2018 24(10): p. 2589–2595. [PubMed: 30184494]
76. Cao Y, Bryan TM, and Reddel RR, Increased copy number of the TERT and TERC telomerase subunit genes in cancer cells. Cancer Sci, 2008 99(6): p. 1092–9. [PubMed: 18482052]

77. Jiang J, et al., Detection of TERC amplification in cervical epithelial cells for the diagnosis of high-grade cervical lesions and invasive cancer: a multicenter study in China, in J Mol Diagn. 2010 p. 808–17. [PubMed: 20864639]

78. Azzalin CM, et al., Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends, in Science. 2007: United States p. 798–801.

79. Feretzaki M, Nunes P. Renck, and Lingner J, Expression and differential regulation of human TERRA at several chromosome ends. RNA, 2019 25(11): p. 1470–1480. [PubMed: 31350341]

80. Bettin N, Oss Pegorar C, and Cusanelli E, The Emerging Roles of TERRA in Telomere Maintenance and Genome Stability. Cells, 2019 8(3).

81. Lopez de Silanes I, et al., Identification of TERRA locus unveils a telomere protection role through association to nearly all chromosomes, in Nat Commun. 2014: England p. 4723.

82. Collie GW, et al., A crystallographic and modelling study of a human telomeric RNA (TERRA) quadruplex. Nucleic Acids Res, 2010 38(16): p. 5569–80. [PubMed: 20413582]

83. Randall A and Griffith JD, Structure of long telomeric RNA transcripts: the G-rich RNA forms a compact repeating structure containing G-quartets. J Biol Chem, 2009 284(21): p. 13980–6. [PubMed: 19329435]

84. Biffi G, Tannahill D, and Balasubramanian S, An intramolecular G-quadruplex structure is required for binding of telomeric repeat-containing RNA to the telomeric protein TRF2. J Am Chem Soc, 2012 134(29): p. 11974–6. [PubMed: 22780456]

85. Deng Z, et al., TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres. Mol Cell, 2009 35(4): p. 403–13. [PubMed: 19716786]

86. Montero JJ, et al., TERRA recruitment of polycomb to telomeres is essential for histone trymethylation marks at telomeric heterochromatin. Nat Commun, 2018 9(1): p. 1548. [PubMed: 29670078]

87. Wang X, et al., Targeting of Polycomb Repressive Complex 2 to RNA by Short Repeats of Consecutive Guanines, in Mol Cell. 2017, 2017 Elsevier Inc: United States p. 1056–1067 e5.

88. Chu HP, et al., TERRA RNA Antagonizes ATRX and Protects Telomeres. Cell, 2017 170(1): p. 86–101 e16. [PubMed: 28666128]

89. Balk B, et al., Telomeric RNA-DNA hybrids affect telomere-length dynamics and senescence, in Nat Struct Mol Biol. 2013: United States p. 1199–205.

90. Balk B, et al., The differential processing of telomeres in response to increased telomeric transcription and RNA-DNA hybrid accumulation. RNA Biol, 2014 11(2): p. 95–100. [PubMed: 24525824]

91. Yu TY, Kao YW, and Lin JJ, Telomeric transcripts stimulate telomere recombination to suppress senescence in cells lacking telomerase. Proc Natl Acad Sci U S A, 2014 111(9): p. 3377–82. [PubMed: 24550456]

92. Porro A, et al., Molecular dissection of telomeric repeat-containing RNA biogenesis unveils the presence of distinct and multiple regulatory pathways. Mol Cell Biol, 2010 30(20): p. 4808–17. [PubMed: 20713443]

93. Redon S, Reichenbach P, and Lingner J, The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase. Nucleic Acids Res, 2010 38(17): p. 5797–806. [PubMed: 20460456]

94. Schoeftner S and Blasco MA, Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II, in Nat Cell Biol. 2008: England p. 228–36.

95. Cusanelli E, Romero CA, and Chartrand P, Telomeric noncoding RNA TERRA is induced by telomere shortening to nucleate telomerase molecules at short telomeres. Mol Cell, 2013 51(6): p. 780–91. [PubMed: 24074956]

96. Farnung BO, et al., Telomerase efficiently elongates highly transcribing telomeres in human cancer cells. PLoS One, 2012 7(4): p. e35714. [PubMed: 22558207]

97. Porro A, Feuerhahn S, and Lingner J, TERRA-reinforced association of LSD1 with MRE11 promotes processing of uncapped telomeres, in Cell Rep. 2014, 2014 The Authors. Published by Elsevier Inc: United States p. 765–76.

98. Deng Z, Campbell AE, and Lieberman PM, TERRA, CpG methylation and telomere heterochromatin: lessons from ICF syndrome cells. Cell Cycle, 2010 9(1): p. 69–74. [PubMed: 20016274]

99. Maicher A, et al., Deregulated telomere transcription causes replication-dependent telomere shortening and promotes cellular senescence. Nucleic Acids Res, 2012 40(14): p. 6649–59. [PubMed: 22553368]

100. Pereira Fernandes D, et al., Long Non-Coding RNAs in Neuronal Aging. Noncoding RNA, 2018 4(2).

101. Gao Y, et al., Regulation of TERRA on telomeric and mitochondrial functions in IPF pathogenesis. BMC Pulm Med, 2017 17(1): p. 163. [PubMed: 29197377]

102. Sinha S, et al., Telomeric Repeat Containing RNA (TERRA): Aging and Cancer, in CNS Neurol Disord Drug Targets. 2015: United Arab Emirates p. 936–46.

103. Ng LJ, et al., Telomerase activity is associated with an increase in DNA methylation at the proximal subtelomere and a reduction in telomeric transcription. Nucleic Acids Res, 2009 37(4): p. 1152–9. [PubMed: 19129228]

104. Hu WL, et al., GUARDIN is a p53-responsive long non-coding RNA that is essential for genomic stability, in Nat Cell Biol. 2018: England p. 492–502.

105. BD A, et al., Targeting noncoding RNAs in disease. 2017 p. 761–771.

106. Matsui M and Corey DR, Non-coding RNAs as drug targets. Nat Rev Drug Discov, 2017 16(3): p. 167–179. [PubMed: 27444227]

107. Li Z and Rana TM, Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov, 2014 13(8): p. 622–38. [PubMed: 25011539]

108. Ling H, Fabbri M, and Calin GA, MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov, 2013 12(11): p. 847–65. [PubMed: 24172333]

109. Sarma K, et al., Locked nucleic acids (LNAs) reveal sequence requirements and kinetics of Xist RNA localization to the X chromosome. Proc Natl Acad Sci U S A, 2010 107(51): p. 22196–201. [PubMed: 21135235]

110. Kasinski AL, et al., A combinatorial microRNA therapeutics approach to suppressing non-small cell lung cancer. Oncogene, 2015 34(27): p. 3547–55. [PubMed: 25174400]

111. Pena-Philippides JC, et al., In vivo inhibition of miR-155 significantly alters post-stroke inflammatory response. J Neuroinflammation, 2016 13(1): 287. [PubMed: 27829437]

112. Nosrati M, et al., Antitumor activity of systemically delivered ribozymes targeting murine telomerase RNA. Clin Cancer Res, 2004 10(15): p. 4983–90. [PubMed: 15297398]

113. Yeo M, et al., Attenuation of telomerase activity by hammerhead ribozyme targeting human telomerase RNA induces growth retardation and apoptosis in human breast tumor cells. Int J Cancer, 2005 114(3): p. 484–9. [PubMed: 15551309]

114. Burger AM, et al., The G-quadruplex-interactive molecule BRACO-19 inhibits tumor growth, consistent with telomere targeting and interference with telomerase function. Cancer Res, 2005 65(4): p. 1489–96. [PubMed: 15735037]

115. Robin JD, et al., Telomere position effect: regulation of gene expression with progressive telomere shortening over long distances. Genes Dev, 2014 28(22): p. 2464–76. [PubMed: 25403178]

CLINICIAN'S CORNER

- An important feature of aging is the progressive accumulation of senescent cells in some tissues, with negative consequences on organ homeostasis and function.
- Senescent cells are characterized by many different hallmarks including a state of indefinite cell cycle arrest and dysregulation of telomere maintenance. Cancer cells are able to overcome replicative senescence by reactivating the telomerase enzyme, thus acquiring aberrant cell proliferation.
- The molecular processes underlying age-associated diseases involve a multitude of noncoding RNAs with crucial roles in cellular processes. New interventions directed at enhancing or suppressing noncoding RNAs are being developed, some of which have already entered human clinical trials. There is mounting interest in RNA-centered strategies to intervene in age-related pathologies such as cancer and neurodegeneration.
- Therapeutic approaches targeting ncRNAs include RNA interference, antisense oligonucleotides and small molecules. Chemical modifications and innovative delivery systems are being developed to improve the stability and effectiveness of these molecules.

OUTSTANDING QUESTIONS BOX

What other cellular processes are regulated by telomeres?

Telomeres are more than just a clock, shortening after each cell division. They protect the genetic information from being truncated during DNA replication, prevent the fusion of chromosomes, and suppress the proliferation of cells with genomic damage. Telomeres may also regulate gene expression through long-distance interaction with other DNA regions. Whether or not ncRNAs are implicated in these *trans*-acting functions of telomeres awaits investigation.

Are there ubiquitous and tissue-restricted telomeric ncRNAs?

We have limited knowledge of the molecular mechanisms by which ncRNAs regulate telomere homeostasis in different cell types. A comprehensive understanding of the molecules and pathways whereby ncRNAs control telomere homeostasis constitutively and in specific tissues will enable more precise interventions.

What disease processes are amenable to therapies targeting telomere-related ncRNAs?

Given that senescent cells have been implicated in many declines and pathologies of aging, interventions aimed at stabilizing or exposing telomeres could prove to be valuable therapeutic tools. With an expanding number of ncRNAs successfully targeted in disease conditions, telomeric ncRNAs are becoming attractive therapeutic targets when senescent cells contribute to disease pathogenesis.

HIGHLIGHTS

- Senescent cells accumulating in aging tissues display indefinite cell cycle arrest, shortened telomeres, and enhanced secretion of pro-inflammatory factors.
- Non-coding RNAs critically regulate gene expression programs in many physiological and pathological processes, including those associated with aging.
- Non-coding RNAs regulate telomere homeostasis, either directly or indirectly, and thereby coordinate molecular processes inherent to cellular senescence and aging.

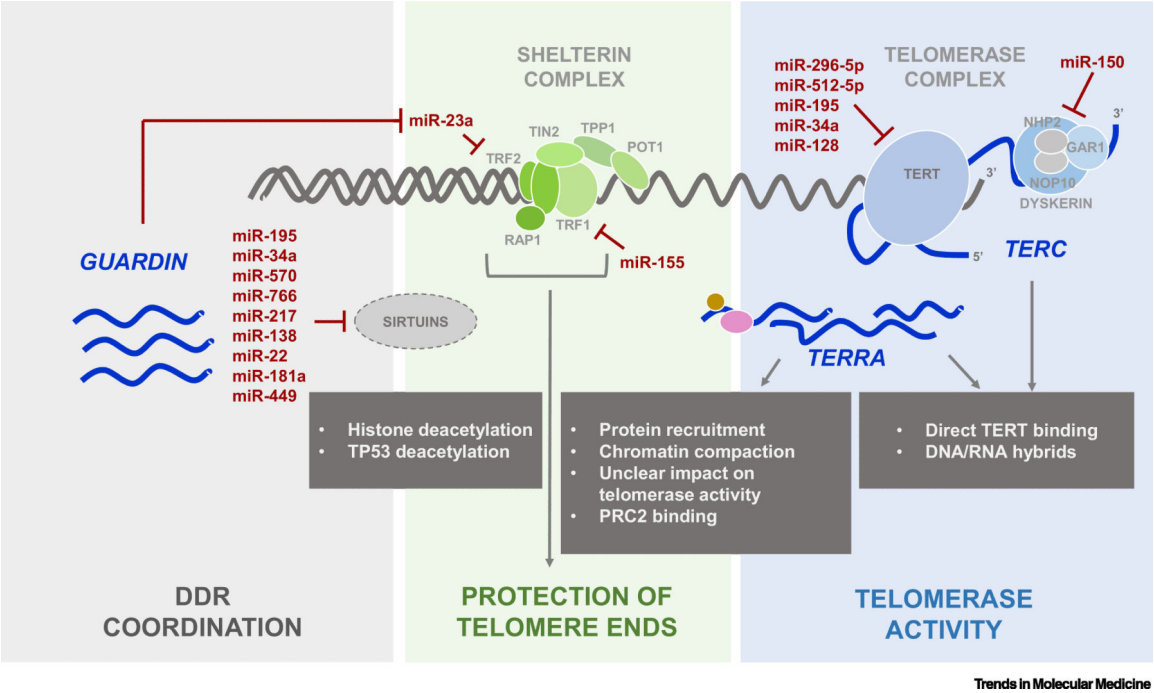

Figure 1.

Schematic indicates the major proteins that protect telomeric integrity (Shelterin complex, green) and length (Telomerase complex, blue), as well as the lncRNAs *GUARDIN*, *TERRA* and *TERC* (blue font) and microRNAs (red font) that modulate telomere homeostasis by integrating the DNA Damage Response (DDR, grey), protecting telomere ends, and controlling telomerase activity.

Table 1.

Main microRNAs identified as regulators of telomere metabolism with a role in senescence and aging.

microRNA	Target mRNAs and pathways	Levels in senescence or aging	Ref.
miR-23a	TRF2	↑	[34, 35]
miR-34a	PNUTS FoxM1/MYC/TERT pathway SIRT1, SIRT6 SIRT7	↑	[38–43]
miR-195	TERT, SIRT1	↑	[44, 45]
miR- 126	Inhibited telomere-TP53-p21-RB and JAK/STAT pathways VCAN (versican), proteoglycan involved in the aging process	↓	[46, 47]
miR-155	TRF1	↓	[48, 49]
miR-22, miR-138, miR-181a, miR-217, miR449, miR-570	SIRT1	↑	[39, 50–55]
miR-26a, miR-145a	SIRT6 and SIRT2 in dysfunctional telomeres	↑	[40]
miR-766	SIRT6	↑	[56]
miR-128	TERT	↑	[57]
miR-296–5p, miR-512–5p	TERT	↑	[58, 59]
miR-150	DKC1	↑	[60]
miR-143, miR-145, miR-146	Can be regulated by expression of ectopic telomerase	↑	[49]
miR-let-7d-5p, miR-let-7e-5p, miR-23a-3p, miR-34a-5p, miR-125a-3p, miR-125a-5p, miR-125b-5p, miR-181a-5p	Increased in senescent fibroblasts, these microRNAs reduced production of cell cycle regulatory factors	↑	[61]