Advances in Long Non-coding RNA TERRA Research (Postprint)

Authors: Fan Yuan, Luo Jia, Gan Mailin, Tan Ya, Zhang Shunhua, Zhu Li

Date: 2018-08-13T00:00:00+00:00

Abstract

Telomeres are specialized structures at chromosome ends that protect chromosomes and are associated with aging and numerous diseases. Long noncoding RNAs are RNAs longer than 200 bp that generally lack coding capacity. TERRA (telomeric repeat-containing RNA) represents a class of long noncoding RNAs transcribed from telomeric repeat sequences. Studies have demonstrated that TERRA participates in the regulation of telomere length, promotes heterochromatin formation, and protects chromosome ends, while its expression correlates with disease and aging. Given TERRA' s crucial role in telomere biology, research on TERRA has emerged as a focal point in telomere-related studies. Currently, a relatively comprehensive understanding of TERRA' s transcriptional regulation and biological functions has been achieved. This review summarizes the biological characteristics, functions, and associations with disease and aging of TERRA, aiming to provide references for future research on TERRA, such as its potential as a therapeutic target for diseases and strategies for delaying aging.

Full Text

Preamble

The Research Advance of Long Non-coding RNA TERRA

Fan Yuan[1], Luo Jia[1], Gan Mailin[1], Tan Ya[1,2], Zhang Shunhua[1], *Zhu Li[1]*
[1]College of Animal Science and Technology, Sichuan Agricultural University, Chengdu 611130, China
[2]Institute of Animal Husbandry and Veterinary, Guizhou Academy of Agricultural Science, Guiyang 550005, China

Abstract

Telomeres are specialized structures at chromosome ends that protect chromosomes and are associated with aging and numerous diseases. Long non-coding RNAs (lncRNAs) are RNA molecules longer than 200 bp that generally lack protein-coding capacity. TERRA (telomeric repeat-containing RNA) is a class of lncRNA transcribed from telomeric repeat sequences. Emerging studies demonstrate that TERRA participates in regulating telomere length, promoting heterochromatin formation, and protecting chromosome ends, with its expression linked to disease and aging. Given TERRA' s crucial role at telomeres, research on TERRA has become a focal point in telomere-related studies. Currently, the transcriptional regulation and biological functions of TERRA have been extensively investigated. This review summarizes the biological characteristics and functions of TERRA and its relationship with disease and aging, providing a reference for future research on TERRA as a therapeutic target for disease treatment and aging intervention.

Keywords: TERRA; telomere; telomere length; chromosomal ends; long non-coding RNA

Introduction

Telomeres are nucleoprotein complexes widely present at the ends of eukaryotic chromosomes. As specialized terminal structures, they prevent chromosomes from being recognized as DNA double-strand breaks (DSBs) and degraded [1]. Telomeric DNA consists of G-rich TTAGGG short double-stranded repeats. The single-stranded repeat sequence forms a G-overhang at the 3' end through the action of 5' -3' exonucleases, which then folds back and inserts into the telomeric double-stranded region to form a protective T-loop structure [2]. The telomere-specific protein complex that binds telomeric DNA is called Shelterin, comprising six proteins: TRF1 (telomeric repeat binding factor-1), TRF2 (telomeric repeat binding factor-2), RAP1 (repressor/activator protein-1), TIN2 (TRF1-interacting protein-2), TPP1 (TINT1/PIP1/PTOP1), and POT1 (protection of telomeres-1). These proteins promote and maintain T-loop structure to protect telomeres [3-5]. Numerous studies have shown that dysfunctional telomeres not only cause cell cycle arrest or apoptosis but are also closely associated with aging and tumorigenesis [6]. Therefore, investigating telomere length regulation is essential.

Current research indicates that regions near telomeres possess transcriptional activity and produce a class of long non-coding RNAs termed TERRA (telomeric repeat-containing RNA) [7]. Studies show that TERRA primarily participates in telomere regulation, with functions including modulating telomere length, protecting chromosome ends, and involvement in cell differentiation and development [8-10]. This review summarizes recent research progress on the biological characteristics and functions of TERRA and its 参与的生物学过程.

Biological Characteristics and Detection Methods of TERRA

1.1 Overview of TERRA

TERRA transcription initiates in the subtelomeric region near chromosome ends and contains UUAGGG repeat sequences. TERRA length is generally considered to range from 100 bp to 9 kb, though recent reports suggest it may exceed 100 kb [7, 11]. RNA polymerase II is the primary polymerase involved in TERRA transcription, though RNA polymerases I and III may also participate [12]. TERRA is widely distributed in most mammalian tissues and, based on nuclear-cytoplasmic localization, is found exclusively in the nucleus, present at the ends of nearly all mammalian chromosomes [13, 14]. However, Le et al. found at the cellular level that only about half of detectable TERRA localizes to telomeres, suggesting TERRA may be involved in biological processes beyond telomere regulation [15].

1.2 Modifications and Processing of TERRA

Like most lncRNAs that possess a 5' cap structure and polyA tail, TERRA also contains a 5' cap and polyA tail. All human TERRA transcripts and most yeast TERRA transcripts have a 7-methylguanosine (m7G) cap structure at the 5' end [16]. While nearly all yeast TERRA molecules contain polyA structures, only approximately 7% of human TERRA molecules have polyA tails, and only polyA-negative TERRA transcripts are associated with telomeric heterochromatin [8, 13, 17]. These findings suggest that different TERRA modifications may determine their regulatory pathways.

Modifications are crucial for TERRA stability. Rat1 is a 5' -3' exonuclease associated with yeast TERRA polyadenylation. Studies show that Rat1 negatively regulates TERRA expression levels through Rap1 (repressor-activator protein 1) and its interacting proteins Rif1 and Rif2 [8]. TRF4, a non-canonical polyA polymerase involved in exosome-mediated RNA degradation, may promote TERRA degradation in Rat1-knockdown cells. Ribonuclease H (RNase H), an enzyme that degrades RNA strands in RNA/DNA hybrids, reduces TERRA expression when overexpressed in budding yeast [18]. Translin and Trax are nucleic acid-binding proteins that regulate nucleic acid degradation and function together. Recent studies found that Translin inhibits TERRA expression levels while Trax maintains them, indicating they can function independently to maintain TERRA homeostasis [19]. Beyond these processing and degradation factors, TERRA degradation primarily occurs through the nonsense-mediated RNA decay (NMD) pathway. Studies show that interfering with mRNA expression of NMD pathway molecules including UPF1, SMG1, and EST1A/SMG6 significantly increases cellular TERRA levels, and ChIP experiments demonstrate these proteins directly interact with TERRA [20].

1.3 Transcriptional Regulation of TERRA

Numerous molecules and pathways have been identified that directly or indirectly regulate TERRA transcription (Figure 1 [Figure 1: see original paper]). Since TERRA is transcribed from telomeres, telomere accessibility affects TERRA transcription. Studies mutating the methyltransferases Suv39h and Suv4-20h found increased TERRA expression, while Schoeftner et al. observed elevated TERRA levels after inhibiting histone deacetylase (HDAC) with trichostatin A, suggesting that increased telomere accessibility promotes TERRA transcription [10, 13].

Many transcription factors directly bind to TERRA promoter sites in subtelomeric regions to promote transcription, including p53 [21], Rb1 [22], CTCF [23], HSP1 [24], and NRF1 [25]. HSP1 is a stress response protein expressed during environmental or heat stress to prevent formation and aggregation of harmful proteins. Koskas et al. found that HSP1 can bind subtelomeric regions, promote TERRA transcription, and maintain telomere integrity, representing a potential novel mechanism for coping with environmental or heat stress [24]. Diman et al. detected activated AMPK/PGC1 pathway in human muscle tissue after exercise, which promoted NRF1 (nuclear respiratory factor 1) binding to subtelomeres to enhance TERRA transcription [25]. Snail1 is a transcription factor that maintains cell stemness. Mazzolini et al. found that overexpression or knockout of Snail1 in mouse mesenchymal stem cells (MSCs) caused corresponding decreases or increases in TERRA expression, indicating Snail1 negatively regulates TERRA transcription [26].

TERRA transcription primarily depends on RNA polymerase II, though some factors influence TERRA transcription through RNA polymerase II. TRF1, a Shelterin component, directly contacts RNA polymerase II as shown by co-immunoprecipitation (Co-IP). Both siRNA-mediated TRF1 knockdown and TRF1 overexpression decrease TERRA expression, suggesting TRF1 may promote TERRA transcription through RNA polymerase II [10]. TLR4 is a receptor protein highly expressed in cancers. Zheng et al. found in liver cancer stem cells that TLR4 inhibits TERRA transcription by preventing RNA polymerase II binding to TERRA transcription regions, thereby promoting proliferation of liver cancer stem cells [27]. FGF2 protein maintains cell stemness. Zeng et al. found that inhibiting the FGF pathway in hESCs decreased TRF1 protein expression and increased TERRA expression, while siRNA-mediated TRF1 interference also decreased TERRA expression, suggesting FGF2 may influence TERRA expression through TRF1 [28]. PAF1 is a conserved transcription elongation factor that binds RNA polymerase II and includes components Cdc73, Paf1, Ctr9, Leo1, and Rtf1 in yeast. Rodrigues et al. found that mutating Paf1 and Ctr9 promoted telomere transcription, while mutating other PAF1 components had no such effect, though whether PAF1 regulates TERRA transcription through RNA polymerase II requires further investigation [29]. Beyond intracellular transcription factors, studies show hormones also affect TERRA expression. Testosterone (TTE) was found

to inhibit TERRA expression and promote telomerase activity in ovarian granulosa cells, though the specific mechanism requires further study [30].

1.4 Detection Methods for TERRA

Northern blot is the most common method for detecting TERRA. With technological advances, quantitative real-time PCR has also been applied to TERRA detection. However, given TERRA' s unique sequence, DNA must be removed during total RNA extraction (e.g., using DNase) to prevent DNA interference and ensure experimental accuracy [31]. FISH probes are also commonly used to detect TERRA. Wang et al. successfully detected TERRA expression levels in peripheral blood leukocytes using FISH probes [32, 33].

Live-cell imaging enables continuous observation and analysis of target molecules. Laprade et al. constructed vectors co-expressing MS2 stem-loop structures fused to TERRA sequences (MS2-pUG6-loxP-kan) and MS2-GFP fusion proteins in budding yeast. The resulting TERRA-MS2 RNA molecules bind MS2-GFP, allowing indirect observation of TERRA expression changes through GFP fluorescence. Laura et al. also observed TERRA expression changes in AGS gastric adenocarcinoma cells using TERRA-MS2 vectors [34, 35].

TERRA Maintains Telomere Length Homeostasis

Telomere shortening compromises the protective function of telomeres. Cells have two pathways to prevent telomere shortening. Telomerase can bind telomeres to extend repeat sequences, preferentially targeting short telomeres [36]. The alternative pathway is alternative lengthening of telomeres (ALT), which primarily induces DNA recombination for homology-directed repair (HDR) through ALT-associated PML bodies (APBs) to maintain telomere length [37, 38].

2.1.1 TERRA Promotes Telomere Shortening

TERRA can promote telomere shortening. The UUAGGG repeat sequence enables TERRA to bind both telomerase RNA (TR) and telomerase reverse transcriptase (TERT), inhibiting telomerase activity and promoting telomere shortening [10, 39]. However, other studies show that the UUAGGG repeats can be bound by hnRNPA1 protein, preventing TERRA from inhibiting telomerase activity [40] (Figure 2A [Figure 2: see original paper]).

Additionally, Farnung et al. constructed exogenous telomeres called transcriptionally inducible telomeres (tiTELs) and found that enhancing transcription to produce TERRA did not inhibit telomere elongation, suggesting that TERRA-mediated telomerase inhibition may be blocked during normal cell cycles [41]. Ku is a telomere-protective protein that prevents telomere recognition and resection by exonuclease 1 (EXO1) [42]. Pfeiffer et al. found that TERRA binds Ku

protein, enabling EXO1-mediated telomere resection. Ku deficiency suppresses this TERRA-induced telomere shortening [43] (Figure 2A).

2.1.2 TERRA Promotes Extension of Damaged Telomeres

TERRA sequences can pair with telomeric DNA to form RNA/DNA hybrids called R-loops [44]. R-loop formation in normal cells disrupts genome integrity and is often accompanied by gene mutations and chromosomal translocations [45, 46]. Therefore, R-loop formation is strictly regulated. The THO/TREX protein complex and ribonuclease H (including RNase H1 and RNase H2) can inhibit R-loop formation [45, 47].

Yu et al. and Balk et al. found that in cells lacking telomerase activity or homology-directed repair, R-loop formation causes telomere damage and rapid cellular senescence. However, in cells with telomerase activity and homology-directed repair, R-loop formation promotes telomere extension [48, 49]. Graf et al. further elucidated this mechanism: Rif2 is a telomere-associated protein that binds Rap1. At long telomeres, Rif2 specifically recruits RNase H2 and Rat1 (a nuclear 5' -3' RNA exonuclease) to degrade TERRA and R-loops before telomere elongation. At critically short telomeres, telomere damage prevents Rif2 binding, allowing TERRA and R-loops to accumulate at shortened regions, activate DNA damage responses, and promote homology-directed repair to extend telomeres and prevent premature senescence [50] (Figure 2B).

TERRA may also promote telomere extension through telomerase. Studies in yeast show that telomere damage increases TERRA expression, and TERRA can serve as a "scaffold" to recruit telomerase and promote formation of telomerase recruitment clusters (T-Resc) to facilitate telomere elongation [51, 52] (Figure 2C). Moravec et al. validated these findings, showing that only polyA-containing TERRA can recruit telomerase and promote its activity to extend telomeres [53].

TERRA Regulates Chromosome Structure and Function

2.2.1 TERRA Promotes Heterochromatin Formation

During certain cell cycle phases, chromosomes condense into heterochromatin to suppress gene expression [54]. Histone H3K9 trimethylation (H3K9me3) is a hallmark of heterochromatin. Studies show TERRA directly binds H3K9me3, histone methyltransferase Suv39h1, and heterochromatin protein 1 (HP1) [55, 56]. Thus, TERRA expression and chromosomal heterochromatinization form a negative feedback loop: during S phase, TERRA recruits Suv39h1 and HP1 to promote H3K9 trimethylation at telomeres, suppressing TERRA transcription and expression [57].

ATRX is a helicase involved in chromatin remodeling that binds H3K9me3 and stabilizes its structure, though this binding is inhibited by H3K4me3 [58, 59]. Porro et al. found that TERRA also binds histone demethylase LSD1 to promote

H3K4me3 demethylation, stabilizing ATRX-H3K9me3 binding and maintaining heterochromatin state [60] (Figure 3A [Figure 3: see original paper]).

2.2.2 TERRA Protects Chromosome Ends

TERRA expression is crucial for chromosome stability. Montero et al. used CRISPR-Cas9 to knock out the TERRA promoter region in the 20q subtelomere of HCT116, HeLa, and U2OS cell lines, causing significant TERRA expression reduction and massive DNA damage response (DDR) induction, demonstrating TERRA' s importance for maintaining chromosome integrity [61].

During telomere replication, exposed single-stranded DNA (ssDNA) is recognized by RPA proteins to activate ATR and induce DDR [62]. Shelterin component POT1 can prevent DDR at telomeres by inhibiting ATR [63]. Studies show hnRNPA1 protein can replace RPA at telomeric ssDNA, and TERRA binds hnRNPA1, enabling POT1 to replace hnRNPA1 and allowing normal telomere capping [64, 65] (Figure 3B).

Beyond cis-regulation of telomeres, TERRA can also act as a trans-regulatory element for other chromosomal regions. Chu et al. used iDRiP proteomics to identify ATRX as TERRA' s primary interacting protein with antagonistic effects. Using CHIRT-seq (combining ChIRP and CHART), they found TERRA and ATRX share the same motif, and TERRA binding positively regulates ATRX target genes [11]. These results demonstrate TERRA can antagonize ATRX through trans-regulation to maintain telomere integrity.

TERRA Participates in Cell Differentiation and Development

Before embryonic stem cell differentiation, TERRA is distributed on all sex chromosomes, but after differentiation, it exists only on one X chromosome in females and the Y chromosome in males [10]. Chu et al. found that in mouse embryonic stem cells, TERRA is primarily transcribed from pseudoautosomal regions (PAR) of sex chromosomes, termed PAR-TERRA. During X-chromosome inactivation (XCI), PAR-TERRA links pseudoautosomal regions and the X-inactivation center (XIC) to silence one X chromosome [66].

Additionally, Marion et al. detected significantly increased telomeric RNA levels in fibroblast-derived induced pluripotent stem cells (iPSCs) [67]. Sagie et al. also found elevated TERRA levels and telomere elongation in iPSCs derived from ICF patient fibroblasts, indicating TERRA did not inhibit telomere elongation through telomerase suppression during this process [68]. Reigviader et al. found TERRA co-localizes with TERT at telomeres during mammalian meiosis, potentially participating in chromosomal changes during meiosis beyond maintaining telomere structure. Comparative studies across species (human and mouse) and sexes revealed species- and sex-specific TERRA expression patterns during meiosis [69]. S. Zeng et al. found TERRA expression gradually

decreased during mouse embryonic stem cell proliferation, leading to increased telomere length and maintenance of stemness [28]. Pre-implantation mouse embryo analysis revealed TERRA production at the 2-cell stage, with expression gradually increasing in a cell cycle-related rhythmic pattern [70]. These results suggest TERRA may regulate telomere length during cell differentiation and development, potentially linking to cell stemness, though this mechanism requires further investigation.

TERRA in Aging and Disease

3.1 TERRA and Aging

Stefano et al. constructed a budding yeast aging model and found that surviving yeast after aging induction had high TERRA expression, suggesting increased TERRA may delay aging [71]. Human aging and health are closely related to telomeres, as abnormal telomere shortening causes premature aging. A study of 10 volunteers undergoing 45-minute cycling endurance exercise found that muscle biopsy samples showed activated AMPK signaling and significantly increased TERRA expression post-exercise, indicating appropriate endurance exercise may maintain telomere integrity and delay aging caused by telomere dysfunction by promoting TERRA transcription [25]. Long-term exposure to environmental persistent organic pollutants causes DNA damage and human aging. Yuan et al. detected significantly elevated TERRA expression in exposed populations compared to controls, but also found a negative correlation between TERRA expression and telomere length in exposed individuals, suggesting chronic pollutant exposure increases TERRA expression to promote aging [72].

3.2 TERRA and Disease

Increasing studies demonstrate close correlations between TERRA expression levels and disease (Table 1). S. Sagie et al. found abnormal TERRA elevation in ICF syndrome patient cells, forming DNA/RNA hybrids with telomeric DNA sequences and causing telomere dysfunction [73]. Wang et al. found a significant negative correlation between peripheral blood leukocyte TERRA expression and polycystic ovary syndrome (PCOS), suggesting TERRA may serve as a therapeutic target for PCOS [30]. Idiopathic pulmonary fibrosis (IPF) is an aging-related lung disease. Studies found TERRA was significantly elevated in IPF patient peripheral blood mononuclear cells, and TERRA interference in cell models improved both telomere and mitochondrial function, indicating TERRA may be a novel biomarker and therapeutic target for IPF [74].

Viral invasion increases TERRA expression, though TERRA's potential function in this context remains unreported [75]. Exosomes are extracellular vesicles that encapsulate non-coding RNAs for information transfer. Wang et al. found TERRA in exosomes secreted by BJ-hTERT cells, termed cfTERRA (cell-free TERRA). Inducing telomere dysfunction in BJ-hTERT cells increased exosomal

cfTERRA levels. Incubating peripheral blood mononuclear cells (PBMCs) with these exosomes stimulated inflammatory cytokine secretion, and cfTERRA was detectable in serum, suggesting cfTERRA may serve as a biomarker for telomere dysfunction caused by early cancer, aging-related inflammation, or viral invasion [76, 77].

Tumor cells generally achieve immortalization by maintaining telomere length through telomerase activity (TA) or alternative lengthening of telomeres (ALT). Theresa et al. overexpressed TERRA in six cancer cell lines and found it reduced colony formation in telomerase-positive but not ALT-positive cancer cells [78]. Vitelli et al. analyzed 23 patients and found TERRA expression significantly correlated with head and neck squamous cell carcinoma invasiveness, with patients having lower tumor TERRA showing worse clinical outcomes [79]. Studies found TERRA binding to indoloquinoline derivative CK1-14 induces TRF2 allosteric changes, causing DNA damage responses at telomeres of U2OS osteosarcoma cells and inhibiting cancer cell proliferation, suggesting TERRA may be a drug target for specific cancers [80]. Naderlinger' s review described that glioma patients with telomerase activity have poor prognosis, and TERRA may serve as a therapeutic target by inhibiting TA activity [81].

Table 1 TERRA involvement in disease regulation

Disease	TERRA-related function
ICF syndrome	Abnormally elevated TERRA expression forms DNA/RNA hybrids with telomeric DNA, causing telomere dysfunction
Polycystic ovary syndrome	TERRA expression significantly negatively correlates with PCOS
Idiopathic pulmonary fibrosis	TERRA interference improves telomere and mitochondrial function
Head and neck squamous cell carcinoma	TERRA expression significantly correlates with tumor invasiveness; patients with lower TERRA have worse prognosis
Glioma	TERRA inhibits cancer cell proliferation when combined with indoloquinoline derivative CK1-14; TERRA may serve as a therapeutic target by inhibiting TA activity

Summary and Outlook

Telomeres are closely related to organismal aging and disease, and TERRA research has become a new breakthrough for understanding telomeres and telomere-related biological processes. TERRA can regulate telomere length through telomerase, which is significant for aging and cancer. However, TERRA' s regulation of telomerase is bidirectional. Future research should focus on determining when TERRA recruits and activates telomerase to extend

telomeres versus when it inhibits telomerase to cause telomere damage. Beyond cis-regulation of telomeres, TERRA can also function as a trans-acting element, and this role requires further elucidation. Current studies on TERRA in aging and disease are mostly preliminary, focusing on disease correlations or cell line experiments. With continuous development of high-throughput sequencing technologies, mining TERRA transcriptional profiles under different disease conditions will be important for future research.

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