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A practical qPCR approach to detect TERRA, the elusive telomeric repeat-containing RNA

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ABSTRACT

Telomeres, the heterochromatic structures that protect the ends of the chromosomes, are transcribed into a class of long non-coding RNAs, telomeric repeat-containing RNAs (TERRA), whose transcriptional regulation and functions are not well understood. The identification of TERRA adds a novel level of structural and functional complexity at telomeres, opening up a new field of research. TERRA molecules are expressed at several chromosome ends with transcription starting from the subtelomeric DNA proceeding into the telomeric tracts. TERRA is heterogeneous in length and its expression is regulated during the cell cycle and upon telomere damage. Little is known about the mechanisms of regulation at the level of transcription and post transcription by RNA stability. Furthermore, it remains to be determined to what extent the regulation at different chromosome ends may differ. We present an overview on the methodology of how RT-qPCR and primer pairs that are specific for different subtelomeric sequences can be used to detect and quantify TERRA expressed from different chromosome ends.

Contents

1. Introduction

Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes. They perform crucial functions as tumor suppressors and they protect chromosome ends from degradation and rearrangements. Telomere functions are mediated largely by the proteins that associate with them. In addition, certain telomere functions are linked to the long noncoding RNA TERRA (for telomeric repeat containing RNA) [1–3]. TERRA transcription starts from promoters in the subtelomeric regions of various chromosomes and proceeds towards chromosome ends [4]. Recent work suggests that TERRA sustains several important functions (reviewed in [2]).

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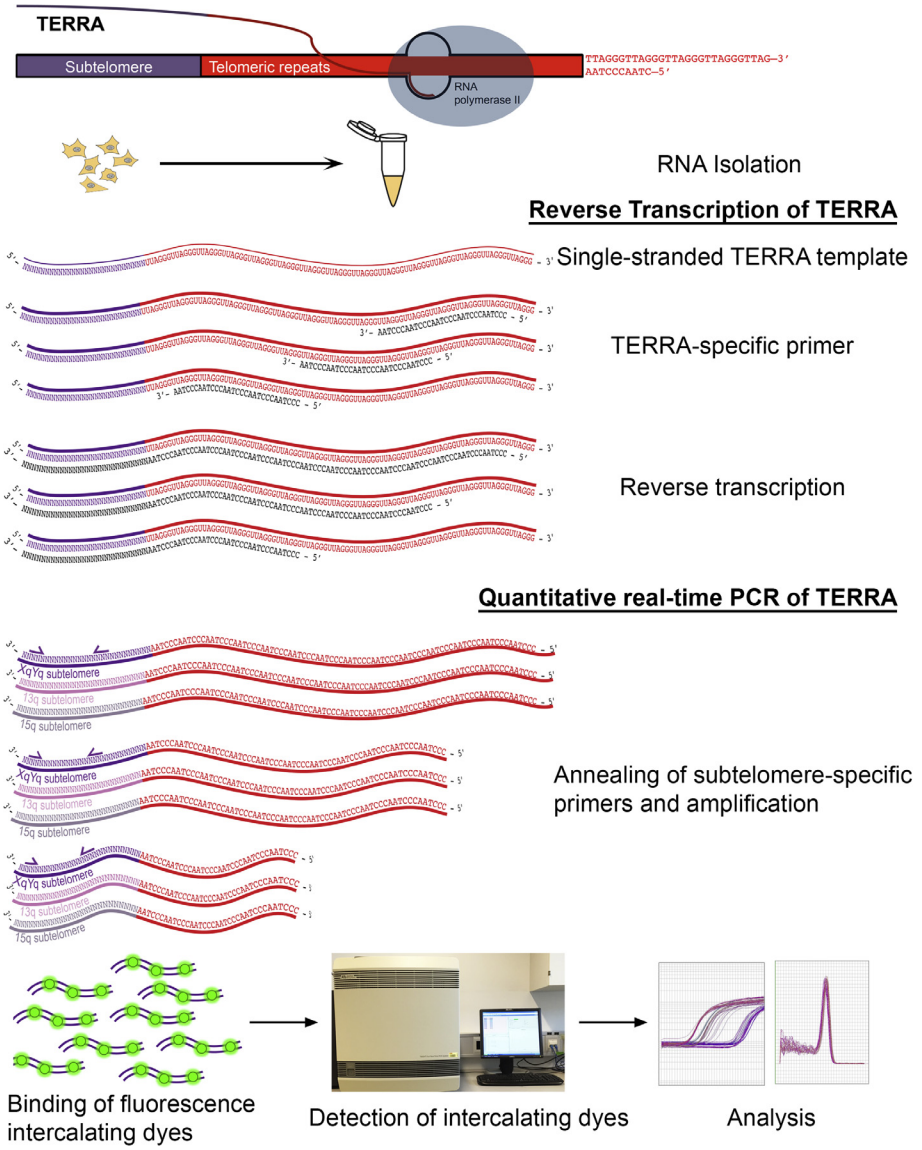

Fig. 1. Schematic representation of TERRA RT-qPCR assay. TERRA synthesis, which is mainly mediated by RNA polymerase II, derives from the subtelomeric region (purple) and extends towards the end of the chromosome into the telomeric repeats (red). A TERRA-specific oligonucleotide comprised of telomeric sequence will anneal and reverse transcribe TERRA from numerous positions in the telomeric tract (red), generating a pool of cDNA molecules with diverse subtelomeric sequences (purple) and length. In quantitative PCR specific primer pairs (purple arrows) amplify the desired subtelomeric sequence from the pool of transcribed TERRA molecules. Fluorescence intercalating dyes bind the double-stranded amplicon, which is detected and analyzed by the RT-qPCR system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TERRA can regulate telomere length through modulation of exonuclease 1 and telomerase [5,6]. TERRA expression is upregulated at damaged telomeres where it sustains recruitment of the chromatin modifiers LSD1 (a lysine demethylase) and SUV39H1 (a histone H3 lysine 9 methylase) thereby enabling DNA end processing [4,7]. TERRA has also been proposed to promote telomere protein composition changes during cell cycle progression. In particular TERRA may favor the binding of the single strand telomeric DNA binding protein POT1/TPP1 to telomeres after their replication during which the single strand DNA binding and replication protein RPA is present at telomeres [8]. Consistent with a role during the cell cycle, human TERRA is expressed during G1 and G2 phases of the cell cycle but is repressed during late S-phase [9]. Finally, recent evidence suggests that TERRA, when engaged into RNA/DNA hybrid structures at chromosome ends, may promote homologous recombination of telomeres in so-called ALT cells (alternative lengthening of telomeres). ALT cells maintain telomeric DNA by recombination instead of telomerase and express high TERRA levels [10,11]. Abnormally high levels of TERRA at telomeres can also interfere with telomere maintenance in human cells as seen in RNA surveillance mutants [1]. In ICF (immunodeficiency, centromeric instability, facial anomalies) patient-derived cell lines which lack DNA methyl transferase 3b [12], subtelomeric CpG islands are undermethylated, which presumably leads to TERRA overexpression in these patients. ICF patients have extremely short telomeres and it remains to be determined whether this phenotype is linked to TERRA overexpression. As opposed to classical analysis of TERRA by Northern blots, RT-qPCR allows accurate quantification of TERRA molecules expressed from individual chromosome ends (Fig. 1). This type of analysis will be most critical to elucidate TERRA regulation and better understand its functions in normal development and its dysfunctions in disease.