Seminars in Cancer Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Tumor viruses and replicative immortality – Avoiding the telomere hurdle

Xinsong Chen, Siamak Akbari Kamranvar, Maria G. Masucci*

Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Keywords:
Tumor virus
Telomere
Genomic instability
Telomerase
Alternative lengthening of telomere

ABSTRACT

Tumor viruses promote cell proliferation in order to gain access to an environment suitable for persistence and replication. The expression of viral products that promote growth transformation is often accompanied by the induction of multiple signs of telomere dysfunction, including telomere shortening, damage of telomeric DNA and chromosome instability. Long-term survival and progression to full malignancy require the bypassing of senescence programs that are triggered by the damaged telomeres. Here we review different strategies by which tumor viruses interfere with telomere homeostasis during cell transformation. This frequently involves the activation of telomerase, which assures both the integrity and functionality of telomeres. In addition, recent evidence suggests that oncogenic viruses may activate a recombination-based mechanism for telomere elongation known as Alternative Lengthening of Telomeres (ALT). This error-prone strategy promotes genomic instability and could play an important role in viral oncogenesis.

1. Introduction

A common property of tumor viruses is their capacity to induce cell proliferation, which establishes a permissive environment for efficient viral genome replication, and expands a reservoir of infected cells that allows long-term persistence in immunocompetent hosts. As reviewed in other sections of this issue, different viruses induce cell proliferation through the expression of multifunctional proteins and regulatory RNAs that target various aspects of cell physiology. These include viral functions that promote the initiation and progression of the cell cycle, inhibit apoptosis, alter the response to different environmental cues and confer the capacity to evade immune responses that could eradicate the infection. While contributing to malignant transformation, these growth-promoting viral products are yet insufficient for the establishment of a fully malignant phenotype. A defining characteristic of malignant cells is their ability to elude regulatory mechanisms that provide an efficient barrier to unlimited cell proliferation. The replication-associated erosion of telomeres and the consequent induction of cell senescence are key component of this barrier. Thus, the path to malignant transformation must involve the engagement of functions that assure the maintenance of functional telomeres. Here we review different strategies by which tumor viruses promote both telomere dysfunction and the maintenance of functional telomeres in proliferating cells. The latter involve the activation of telomerase, the unique enzyme that maintains telomere length, but also the triggering of telomerase-independent mechanisms for telomere elongation that are associated with a high degree of genomic instability and could play an important role in tumor initiation and progression.

2. Telomeres and the maintenance of telomere integrity

The linear DNA molecules of eukaryotic chromosomes are capped by a DNA–protein complex known as the telomere that is composed of a double stranded array of the 5′-TTAGGG-3′ repeat terminating in a 3′ single-stranded overhang [1], and a six-subunit protein complex known as the shelterin [2] (Fig. 1). The telomere solves two problems that are inherent to the edges of linear genomes: it distinguishes the chromosome ends from double strand DNA breaks, which avoids the activation of unwanted DNA damage responses (DDR); and provides a buffer to the imprecise replication of the ends, which prevents the loss of essential genetic material during cell proliferation.

The telomere conceals the chromosome end in a looped structure known as the T-loop that is formed by invasion of the 3′ single-stranded overhang into the double-stranded region [3]. The T-loop is stabilized by the shelterin complex that includes three DNA-binding proteins: the telomere repeat binding factor 1 and 2 (TRF1 and TRF 2) [4], and protection of telomeres 1 (POT1) [5] and their interacting partners TPP1 (adrenocortical dysplasia

* Corresponding author at: CMB, Karolinska Institutet, Berzelius väg 35, S-17177 Stockholm, Sweden. Tel.: +46 8 52486755.
E-mail address: maria.masucci@ki.se (M.G. Masucci).

1044-579X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcancer.2014.01.006

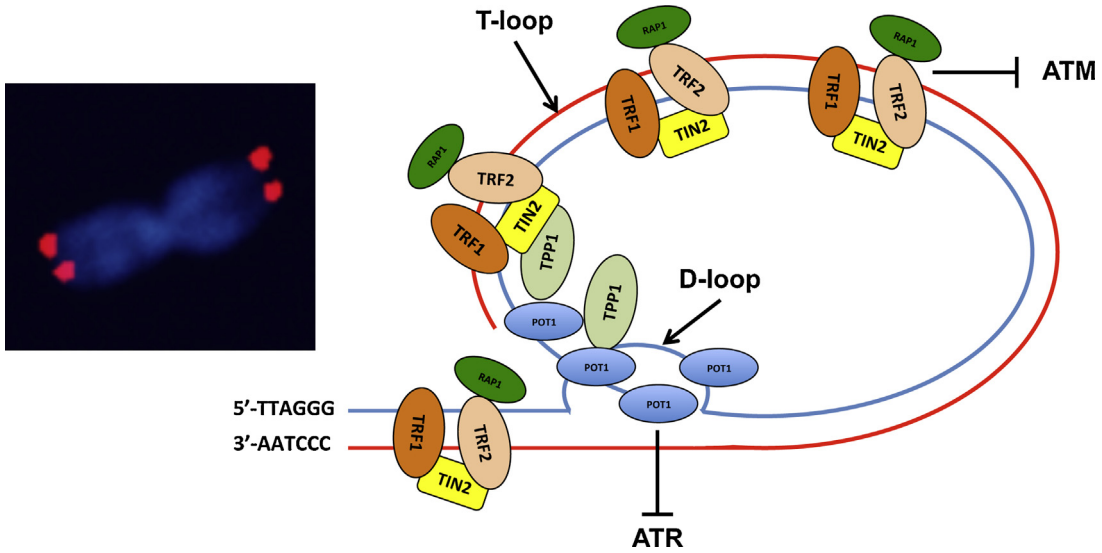

Fig. 1. Structure of the telomere. Human telomeres consist of multiple copies of the TTAGGG sequence, with a G-rich leading strand and a C-rich lagging strand. The G-strand extends in the 3′ direction, forming the G-overhang. The double- and single-stranded repeats are covered by the shelterin complex consisting of the double-stranded Telomeric Repeat-binding Factor-1 and -2 (TRF1 and TRF2), the TRF2-interacting Repressor and Activator Protein-1 (RAP1), the bridging molecules TRF1-Interacting Nuclear protein 2 (TIN2), the TIN2 and POT1-interacting Protein (TPP1) and single strand DNA binding Protection Of Telomeres 1 (POT1). Shelterin members interact with many other factors that transiently localize at telomeres, frequently in a cell-cycle-dependent manner. These factors stabilize a protective structure known as the T-loop that is generated by invasion of the single-stranded G-overhang into the double-stranded TTAGGG repeats. Invasion effectively sequesters the G-overhang, which distinguishes the chromosome ends from double-strand breaks. The ATM-dependent signaling cascade is inhibited by TRF2 while POT1 inhibits ATR-dependent signaling.

protein homolog 1) [6], TIN2 (TRF1-interacting protein 2) [7] and RAP1 (TRF2-interacting repressor and activator protein 1) [8]. The shelterin subunits have different functions [9]. TRF2 plays a major role in preventing the recognition of telomeres as damaged DNA. Disruption of TRF2 activates an ataxia telangiectasia mutated protein (ATM)-dependent DDR that causes end-to-end telomere fusions [10], while simultaneous loss of TRF2 and the DNA double-strand break repair factor Ku70 activates homologous recombination-dependent telomere sister-chromatid exchange (T-SCE) [11]. The capacity of TRF2 to repress homologous recombination is dependent on interaction with RAP1 since expression of a RAP1-binding mutant cannot prevent T-SCE in TRF2/Ku70 deficient cells [12]. TRF1 is involved in the regulation of telomere replication and its disruption is associated with telomere elongation and significantly increased levels of sister-telomere associations [13]. POT1 is recruited to the single-stranded telomere overhang through sequence-specific binding and *via* high affinity interaction with TPP1. Independent disruption of either POT1 or TPP1 leads to an Ataxia Telangiectasia and Rad3-related (ATR)-dependent DNA-damage response and telomere fusions [14,15]. The TPP1/POT1 complex enables increased telomerase processivity *in vitro* [16], and TPP1 might be directly involved in the recruitment of telomerase to the telomeres [17]. TIN2 bridges the single and double stranded DNA binding complex and stabilizes the shelterin by simultaneously binding to TPP1, TRF1 and TRF2 [18,19].

The organization of telomeric chromatin also plays an important role in preserving telomere integrity [20]. Similar to the rest of chromosomal DNA, the telomeres of higher eukaryotes are wrapped into nucleosomes but these are tightly packed and separated by shorter linker regions, resulting in an unusual chromatin structure [21–23]. Maintenance of this structure is regulated by both histone post-translational modifications and by the incorporation of histone variants [24]. In mammalian cells, telomeric chromatin contains epigenetic markers of heterochromatin similar to those found in pericentric heterochromatin, such as trimethylated and hypoacetylated histones H3 and H4 [25–27]. Knockout of histone and DNA methyltransferases results in defective telomere function, aberrantly increased telomere length, and chromosomal instability [26,28], suggesting that the repressive markers are essential for telomere length maintenance and structural integrity. Another layer of complexity is added by the incorporation of histone variants. Canonical histone genes are clustered in repeated arrays and are almost exclusively transcribed during the S-phase whereas histone variant genes are generally present in single copy and are expressed throughout the cell cycle. Recently, mouse and human telomeres were shown to contain the histone variant H3.3 [29] that is deposited at telomeres by the chromatin remodeling complex ATRX in cooperation with the histone chaperone DAXX [30,31]. Knockdown of ATRX by RNAi causes telomere dysfunctions in mouse embryonal stem cells [30] and is associated with up-regulation of a large non-coding telomeric repeat-containing RNA (TERRA) [29]. TERRA regulates telomere length by binding to and inhibiting the activity of telomerase [32] and by promoting exonuclease-1 mediated resection of the telomere ends [33].

Telomere sequences are lost during each round of cell division due to inability of the replicative polymerase to completely duplicate linear DNA (the so called ‘end replication problem’). This replicative limitation causes progressive telomere shortening and is ultimately associated with organismal aging [34]. Therefore, the second major function of the telomere is to prevent the loss of genetic information and provide a means for length maintenance in replicating cells. The lengthening function is fulfilled by the enzyme telomerase, a reverse transcriptase complex that uses a short RNA as template to direct the addition of telomeric repeats onto the chromosome ends [35]. The catalytically active telomerase complex purified from human cells is a dimer minimally composed a telomerase reverse transcriptase (TERT) subunit, a template-containing telomerase RNA (TER), and the small nucleolar ribonucleoprotein (snoRNP) family member dyskerin (DKC1) [36] (Fig. 2). The enzyme is normally active in human stem/progenitor cells and germ-line cells, and in a subset of somatic cells (*e.g.*, activated lymphocytes) [37], whereas activity is silenced or kept at very low levels in most somatic cells [38]. The regulation of telomerase activity is primarily achieved at the level of transcription

Please cite this article in press as: Chen X, et al. Tumor viruses and replicative immortality – Avoiding the telomere hurdle. Semin Cancer Biol (2014), http://dx.doi.org/10.1016/j.semcancer.2014.01.006