MINI-REVIEW

Shelterin Proteins and Cancer

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Abstract

The telomeric end structures of the DNA are known to contain tandem repeats of TTAGGG sequence bound with specialised protein complex called the "shelterin complex". It comprises six proteins, namely TRF1, TRF2, TIN2, POT1, TPP1 and RAP1. All of these assemble together to form a complex with double strand and single strand DNA repeats at the telomere. Such an association contributes to telomere stability and its protection from undesirable DNA damage control-specific responses. However, any alteration in the structure and function of any of these proteins may lead to undesirable DNA damage responses and thus cellular senescence and death. In our review, we throw light on how mutations in the proteins belonging to the shelterin complex may lead to various malfunctions and ultimately have a role in tumorigenesis and cancer progression.

Keywords: Shelterin proteins - telomeres - telomerase - tumorigenesis

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Introduction

Shelterin is a protein complex at the end of the chromosomes, shaped by six telomere-specific proteins and functioning as a safeguard for the chromosome ends (Huawei et al., 2008). The name "shelterin" is derived by analogy to other chromosomal protein complexes such as cohesin and condensin. Proteins TRF1, TRF2 and POT1 directly recognise the TTAGGG repeats and are interconnected by additional three subunits TIN2, TPP1 and RAP1, enabling cells distinguish telomeres from other sites of DNA damage in the genome (Titia de Lange, 2005). Shelterin blocks three major pathways that can damage telomeres viz., activation of ATM and ATR kinases; Non homologous end joining (NHEJ) and Homology directed repairing (HDR) (Kibe et al., 2010). If the protective capability of shelterin is lost, telomere sites get exposed to DNA damage and repair proteins alike, threatening genome integrity.

Telomeric Repeat Binding Factor 1 - TRF1

Telomeric Repeat Binding Factor 1 (TRF1) is one of the proteins of shelterin complex comprising of 439 amino acid residues (UNIPROT-P54274 (TERF1_HUMAN)). It is involved in binding to the duplex array of TTAGGG repeats at the telomeres (Judith et al., 2001; Michael and Charles, 2013). It has specific conserved domains which assist in the formation of a stable TRF1-TRF1 homodimeric structure along with the two myb-domains of the homodimer which help in a stable interaction with the duplex DNA at the telomere by binding to it in a specific bending angle of approximately 120degrees (Alessandro et al., 1997; Huaweiet al., 2008). Furthermore, research revealed that TRF1 forms homodimer prior to its association with the telomeric double strand DNA (Lingjun et al., 2011).The stability and telomere binding affinity is negatively influenced by the interactions of TRF1 with Nucleostemin(NS) and positively influenced by that of GNL3L (guanine nucleotide binding protein-like-3-like). GLN3L not only enhances the stability of homodimer but also prevents ubiquitylation and degradation. While on the other hand binding of NS yields a disrupted homodimer and diminished affinity to bind to TTAGGG repeats at the telomere (Lingjun et al., 2011).

TRF1 plays an important role in the assembly of the shelterin complex by binding with Telomere Repeat Binding Factor2 (TRF2) via TRF-1 Interacting Nuclear Protein-2 (TIN2) (Huawei et al., 2008; Raffaella and Diego, 2011). Any distortion in TRF1 structure owing to alterations in nucleotide/protein sequences directly affects its association with the other proteins of the shelterin complex including disruption of its dimerization (Louise et al., 2001). On TRF1 dimerization disruption, DNA binding is changed and hence the TTAGGG repeats are not protected, all attributable to an erroneous assembly of the shelterin proteins (Jason et al., 2011). The telomere ends when unguarded by the shelterin proteins get exposed and vulnerable to DNA repair mechanisms and presumably to telomerase action, thus resulting in telomere attrition, vast number of structural and molecular changes due to non-specific repairs and/or cellular senescence. This challenges the stability of genome leading to early hallmarks of cancer.

The major function of TRF1 is to negatively regulate the telomere length by suppressing the telomerase activity

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(Bas and Titia, 1997). It is also known to interact with the telomere during interphase and mitosis (Masafumi et al., 2002; Xiao et al., 2003). Absence of TRF1 or a non-functional mutant TRF1 renders inability to regulate the telomere length. TRF1 activity is also significantly associated with Ataxia-telangiectasia-mutated (ATM) pathway, a pathway important as sensor during DNA damage. Activation of ATM due to DNA damage response results in phosphorylation of TRF1 by it and thus negative regulation of TRF1 (Shuji et al., 2001; John and Xu-Dong, 2012). Abrogation of TRF1 causes blockage of ATM-TRF1 interaction leading to non-recruitment of P53 and CHK2 which in turn does not allow replication fork stalling. Failure to stall the replication fork at DNA damage leads to unchecked cell cycle and hence mutations, the consequence being aberrant cell division and genomic instability (Jorunn and Sigridur, 2005; Qian and Jiandong, 2010).

TRF1 actively interacts with tankyrases TANK1 and TANK2 which positively regulate the telomere length. It does so by ADP-ribosylating TRF1 and releasing it from the telomeric end and thus allowing room for telomerase to act upon it (Jill and Titia, 2007). In addition, study done by Yoon and In (2009) demonstrates that RLIM (RING H2 zinc finger protein with intrinsic ubiquitin ligase activity) is involved in down-regulation of TRF1 by causing ubiquitin-mediated degradation and eventual maintenance of the telomeric length (Yoon and In, 2009). Over expression of tankyrases disrupt TRF1 function and thus exposes telomeric ends to telomerase activity altering telomere length maintenance which ultimately is an important factor in cancer progression.

TRF1 is also known to associate with Ku proteins, a major component of NHEJ (Non-homologous end joining) pathway (Huichen et al., 2003; Karel et al., 2006). Any disruption in TRF1 activity causes activation of Ku proteins which recognizes the double strand breaks (DSBs) in telomere end and exerts the error prone DNA repair mechanism, thus activating the NHEJ pathway resulting in chromosome fusions, terminal deletions, accumulation of mutations and massive multi telomeric signals (MTS).

Telomeric Repeat Binding Factor 2- TRF2

Telomeric Repeat Binding Factor 2 (TRF2) is an important protein of shelterin complex comprising of 542 amino acid residues (UNIPROT-Q15554 (TERF2_HUMAN)). It is involved in binding to the duplex array of TTAGGG repeats at the telomeres. TRF2 binds to the double stranded/single stranded DNA junction (Paula and Maria, 2010; Fei et al., 2012). Like TRF1, it is also a homodimer with C-terminal myb-domains for binding directly to the DNA. It is ubiquitously expressed, bound specifically to duplex TTAGGG repeats in vitro and localised to all human telomeres in metaphase chromosomes. TRF 2 shares an architectural similarity with TRF1 but it still differs from TRF1 in that its N-terminus is basic rather than acidic and is much more conserved than TRF1 (Jason et al., 2012).

TRF2 mediates its role partly by the formation of heterodimer with the telosome component RAP1 which contain a myb-domain. Expression of mutant forms of TRF2 has been shown to induce a proliferative arrest with the characteristics of senescence. Chromosomal end fusions and senescence in primary human cells may be caused by loss of TRF2 from shortened telomeres (Bas et al., 1998). When compared with normal mucosa tissues, the expression of TRF1, TRF2 and TIN2 proteins was found to be significantly higher in precancerous lesions, gastric cancer and gastric cancer with lymph node metastasis (GCLM) (Hua et al., 2010). It is shown to play a central role in telomere maintenance and protection against end to end fusion of chromosomes.

It recruits DNA damage sensing and DNA repair proteins including the shelterin complex to the telomeric region (Jun et al., 2013). It also can be recruited to intra satellite double strand breaks when the damage is very high. Without its protective act telomeres are no longer hidden from the DNA damage surveillance and the chromosomal ends are inappropriately processed by different DNA repair mechanisms. Along with the DCLRE1B/Apollo (having 5'-3'exonuclease activity thus involved in protection of telomeres against NHEJ DNA repair), it plays an important role in the formation of the telomeric loop (T loop) that generates 3' single stranded overhang at the leading end telomeres and stabilises it. The T-loops thus formed protect chromosomal ends from degradation and repair. Hence, it is required both to recruit DCLRE1B/Apollo to telomere and activate the exonuclease activity of the same (Bas et al., 1998; Titia, 2005; Jing et al., 2010; Liu et al., 2013). TRF2 recruits TERF2IP/RAP1 to telomeres hence participating in repressing the homology directed repair (HDR), which can affect the telomere length. TRF2 interacts with a number of factors involved in DNA repair, which also have a role at telomeres. Many of them are mutated in human chromosomal instability syndromes characterised by premature aging and short telomeres.

The known TRF interacting DNA repair factors include WRN (mutated in Werner Syndrome), BLM(mutated in Bloom Syndrome), Ku86 involved in DNA double strand break repair by NHEJ. Other factors like ERCC1/XPF endonuclease (mutated in Xenodermapigmentosum) involved in nucleotide excision repair(IVER), the MreII/NbsI/Rad50 (M-N-R) complex(mutated in Nigimegen breakage syndrome and involved in DNA double strand break repair) as well as the PARP1 and PARP2, poly(ADP) ribosylases are involved in base excision repair(BER) also interact with TRF2. ATM a major sensor in DNA damage signalling interact with TRF2 so that the telomere protein does not allow any action of ATM on the telomere and prevent repair of telomere.

TRF2 itself has a significant role in human diseases. High levels of TRF2 have been observed in a number of human tumours (lung, liver and gastric cancer) and human skin carcinomas. Overexpression of TRF2 in the skin of mice has been shown cause a rapid and dramatic loss of telomeres in the presence of normal telomerase levels. Overexpression of telomerase in these mice was unable to prevent telomere shortening underlining the role of this protein in telomere integrity. TRF2 mice represent a

model of premature skin aging by short telomeres in the presence of normal telomerase levels (Jill RD et al., 2007). In another study group, demonstration of TRF2 as an important telomere protein was determined by comparing two individual mouse models: - (i) the telomerase deficient mouse (resistant to carcinogenic treatment), (ii) TRF2 transgenics (tumour prone). TRF2 transgenics is the wild type for telomerase genes and therefore can potentially up regulate telomerase and elongate short telomeres (thus continuing cell division). The telomerase deficient mouse cannot reactivate telomerase because they are deficient of TERC genes (therefore short telomeres will activate, DNA damage response will act as tumour suppressing genes). An alternative explanation for this can be that TRF2 overexpression might cancel the ATM dependent DNA damage response caused by short telomeres, hence allowing the accumulation of chromosomal aberrations and continuation of cell growth. TRF2 overexpression leads to increased chromosomal instability, which might be a result of a combination of telomere uncapping due to critical telomere shortening and a defective NER pathway hence, increased level of TRF2 can promote tumorigenesis and can progress number of human cancers (Munoz et al., 2006).

The XPF/ECCR1 complex is involved in the repair of UV induced DNA lesions through the nucleotide excision repair (NER) pathway, as well as in DNA crosslink repair. An interaction between TRF2 and XPF/ERCC1 and its localisation to telomeres has been demonstrated. XPF/ERCC1 is shown to be able to degrade the single strand G strand overhang at telomeres in the cells with mutant TRF2, proving that TRF2 can regulate the XPF/ERCC1 activity. In particular, increased TRF2 may sequester most XPF/ERCC1 at telomeres resulting in abnormal telomeric degradation and results in defective NER at DNA lesion elsewhere in the genome at the same time.

TRF1 Interacting Nuclear Protein 2 - TIN2

TRF1 interacting nuclear protein-2 comprises of 451 amino acids (UNIPROT-Q9BSI4 (TINF2_HUMAN)). The 196 residues at the N-terminal of TIN2 are responsible for mitochondrial localization and also for interaction with TPP1 in both cytoplasm and nucleus (Chen et al., 2007; Liu et al., 2004). Localization of TIN2 in mitochondria is responsible for bringing down the ATP production and increasing reactive oxygen species (ROS) in case of aging, whereas absence of TIN2 in mitochondria will increase the ATP production and bring down ROS which could lead to increased metabolic proliferation of the cell and in long run can lead to uncontrolled proliferation and instability in cell (Sullivan et al., 2012). Disruption of this region can reduce localization of TIN2 towards nuclear/telomere while promoting its cytoplasmic/mitochondrial targeting. Studies suggest that silencing of TIN2 cannot allow any possible interaction with TPP. For TIN2-TPP1 interaction an intact N-terminal is very essential and deleting just the first 18 amino acids is sufficient to terminate the interaction and prevent nuclear localization of TIN2 (Liuh-Yow et al., 2012).

TIN2 is a central component of shelterin that not only connects TPP1/POT1 to the other shelterin components but also stabilizes TRF1 and TRF2 on the duplex telomeric repeat array (Lei et al., 2004; Ye et al., 2004; Kim et al., 2004). The function of TIN2 is of particular interest since reports suggest that it is found to be mutated in a subset of Dyskeratosiscongenital (DC) patients (Savage et al., 2008) a spectrum of condition termed as telomeropathies. TIN2 mutants elicit the phenotypes associated with POTa/b deletion (Liuh-Yow et al., 2012) which leads to increase in the (single strand) ss-TTAGGG repeats and unprotected ends. Another prominent phenotype due to loss of TIN2 is polyploidization through endo-reduplication, a phenomenon known to cause leading and lagging-end telomeres fusion contributory to fused chromosomes and translocations (Hockemeyer et al., 2006; Davoli et al., 2010; Kaori et al., 2011). A substantial number of fusion are generated in TIN2 deficient cells, among which sister telomere fusion are prominent indicating that TIN2 loss resulted in de-protection of both leading- and lagging-end telomeres. This may be because the loose ends of the telomere are left unwatched and hence overall become vulnerable to DNA repair proteins and other alternations of chromosomes. TIN2 deletion also results in activation of DNA damage response by accumulation of 53BP1 (p53-binding protein 1) at telomeres, proliferative arrest and phosphorylation of Chk1 and Chk2. Hence activation of ATM and ATR occurs at telomeres lacking TIN2resulting initiation of repair mechanisms and in turn lead to Alternating telomere lengthening (ALT) pathways by sister chromatid exchange at telomere end in a homologous matter manner. Contradictory to above study, TIN2 is also known to contribute to TRF2-mediated repression of ATM signalling. This has been observed in TIN2 knockout cells to study the diminished TRF2 function in such cells (Liuh-Yow et al., 2012).

Protection of Telomeres Protein-1 POT1

POT1 (Protection of telomeres protein 1) is a protein with a sequence length of 634 amino acids (UNIPROT-Q9NUX5 (POTE1_HUMAN)) and is one of the six proteins of shelterin complex. The mouse genome contains two POT1 orthologs, viz., POT1a and POT1b (Paula et al., 2010). Increased DNA damage focused at telomeres, endo-reduplication and early induction of senescence was seen in cells with double knockout of these genes (He et al., 2006; Hockemeyer et al., 2006). POT1a is involved in inhibiting DNA damage repair at telomere, while Pot1b has the ability to regulate the amount of single-stranded DNA at telomeres in a telomerase-independent manner (Hockemeyer et al., 2006). Deletion of Pot1a resulted in early embryonic lethality, while Pot1b-deficient mice can survive to adulthood and only shows degenerative phenotypes like DC patients - dominantly skin hyper-pigmentation and bone marrow failure, when generated in a telomerase-haplo-insufficient background (He et al., 2009). POT1 plays a critical role in protecting the telomere from DNA damage signalling, a protective effect needed for normal telomere functioning. Kentaro and co-workers demonstrated that the expression of Pot1a in Hematopoietic stem cells (HSC) significantly

decreased with age in in vitro culture. Moreover in vitro POT1a knockouts of HSCs showed increased level of DNA Damage Response (DDR) and significantly reduced long-term repopulation (LTR) activity of HSCs. Opposing to this POT1 also binds to 10 nucleotide single-stranded TTAGGGTTAG sequence and prevents the inappropriate activation of (ATM) and Rad3-related kinase at the 3aaa telomeric overhang to ensure that the chromosome end is not recognized as DNA damage (Denchi and de Lange, 2007). In addition, POT1 acts as a regulator of telomerase-dependent telomere length and it can help telomere to form D-loop structure to stabilize telomere as well (Qingqing et al., 2014). Silencing of either POT1 or TPP1 by RNA interference induces telomere lengthening and chromosomal instability (Kelleher et al., 2005), indicating their role in regulating telomerase access to the overhang (Lei et al., 2004). As POT1 plays an important role in telomere protection in relation to telomere elongation and cell immortality, it has been recognized as a promising drug target for cancer treatment (Xiao et al., 2012). POT1 protects single stranded telomeric DNA by interacting with TPP1 via heterodimerization (Baumann et al., 2002) and it protects telomeres by contributing to the formation of t-loop formation in association with TRF2 and regulates the nucleolytic process responsible for 3'overhang formation (Greider, 1999; Hockemeyer et al., 2005). Deletion of the pot1 gene leads to rapid loss of telomere sequences, chromosome mis-segregation, chromosome circularization, and chromosome end-to-end fusion (Baumann and Cech, 2001) which in turn has a major implication in genomic instability. Thus, inhibition of human POT1, either by expression of a dominant negative form or by siRNA knockdown leads to a DNA damage response and telomere dysfunction contributing to progression of cancer when coupled with mutations in tumor suppressors or oncogene (Hockemeyer et al., 2005; Veldman et al., 2004). There is a strong association of POT1 with plasma cell disorders like monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) along with other shelterin proteins, suggesting POT1 as another possible molecular target to design new therapeutic strategies (Julieta et al., 2014).

POT1-TIN2 Organizing Protein- TPP1

The POT1-TIN2 Organizing Protein (TPP1, also known as PTOP, TINT1 or PIP1) is a protein of telosome complex involved in the regulation of chromosomal ends. It is composed of 544 amino acids (UNIPROT ID-Q96AP0 (ACD_HUMAN)). It links TIN2 and POT1 while chiefly interacting with POT1 (Jeffrey et al., 2004; Tatsuya et al., 2010). TPP1 has very significant structural role in the assembly of the shelterin proteins. It conjoins TIN2 and other shelterin units with POT1 thus signifying that any mutation resulting in structural moderations in TPP1 accounts for tapered assembly of the shelterin complex (O'Connor et al., 2006; Liuh-Yow et al., 2007). Such an event is responsible for activation of elicited DNA damage response and thus cellular senescence.

TPP1 along with POT1 performs a dual function at the telomeres viz. - it restricts the exposure of telomeric TTAGGG repeats to DNA damage response and the telomerase activity while on the other hand, it also allows binding of telomerase to the telomeric region through TPP1-OB fold domain albeit there is insufficient POT1 occupancy at the telomeres (Feng et al., 2007; Xiaoping et al., 2009; Tatsuya et al., 2010; Yi et al., 2012; Franklinet al., 2012). The TPP1-OB fold is an oligonucleotide binding fold domain which permits interaction with TERT hence recruiting telomerase to the chromosome ends (Franklin et al., 2012; Xin et al., 2013). Also, the OB-fold domain of TPP1 is involved in homodimerization which is known to be regulated by the Protein kinase B. Thus, its inhibition contributes to telomere dysfunction accompanied by a decline in TPP1 and POT1 recruitment (Xiaolan et al., 2007).

TPP1 is also known to interact with ATM pathway and eventually any mutation in TPP1 resulting in a non-functional protein unit causing an elicited P53 dependent growth arrest, DNA damage response via ATM and ATR pathways hence ultimately stimulating chromosomal instability and large accumulation of mutations leading to tumorigenesis and cancer. In certain cases cellular senescence also occurs through the same pathway and a massive cell death is presumed to occur at local tissue site (Moretti et al., 1994). Removal or any mutation in TPP1 and thus disrupted TPP1-POT1 complex leads to telomere dysfunction due to initiation of NHEJ pathways (Rekha et al., 2010).

TPP1 also functions to enhance POT1 DNA binding activity by several folds. Therefore, abrogation of TPP1 allows for only partial DNA binding capacity of POT1 thus ultimately resulting in diminished functionality of the shelterin complex (Feng et al., 2007). A reduced protection from the shelterin complex at the telomeres eventually leads to elicited DNA damage response, recruitment of P53 and other mechanisms because of which tumorigenesis and cancer progression have a high risk of occurrence.

Human Repressor Activator Protein 1-RAP1

RAP1 also known as TRF2 interacting protein of the telosome complex, composed of 399 amino acids (UNIPROT ID- Q9NYB0(TE2IP_Human),protects the telomere ends from the attacks by the DNA repair mechanisms like other proteins of the complex but by different mechanisms (Fei et al., 2012). RAP1 forms a complex with TRF2 and this binding is necessary for the binding of RAP1 to telomeres. Despite its telomeric location it was shown that RAP1 is expendable for telomere capping but prevents telomere recombination and fragility. Hence, RAP1 is not a telomere protective protein, in contrast to the other shelterin proteins. Although RAP1 deletion did not show a dramatic effect on the length of the telomeres of immortalised mouse embryonic fibroblasts (MEFs), mouse epidermis lacking RAP1 did show 26% reduction in mean telomeric length and showed an increase in H2AX (Gamma irradiated nucleosomal histone protein- it is an assay to detect DNA double-stranded breaks) DNA damage foci, indicating its probable role in

telomerase regulation (Titia de Lange, 2005). A function of mammalian RAP1 in protecting telomeres from NHEJ mechanisms has been observed in the context of severe telomere uncapping induced by TRF2 dysfunction (Paula and Maria, 2010). RAP1 is also shown interact with Silent Information Regulator proteins - Sir3 and Sir4. Its interaction with Sir3 can be observed in vitro when no other yeast proteins are present. Native SIR Proteins interact with the carboxyl terminus of RAP1. It was also shown that the mutated forms of Sir3 and Sir4, increases transcriptional activation by a repressor enzyme LexA and RAP1 hybrids. Mutations were identified in the carboxyl terminus of RAP1 that would abolish the interaction with SIR3. These mutations were introduced in the native RAP1 protein, and were seen to cause corresponding defects in the silencing at both HMR and telomere. Thus, it can be proposed that RAP1 plays a significant role in the initiation of transcriptional silencing by interacting with a complex of SIR proteins. Model studies have also shown that SIR3 and SIR4 play a structural role in maintaining silent chromatin. (Moretti et al., 1994).

Conclusions

All telomere proteins when damaged, at one point or other; over expressed or under expressed do have the potential to recruit the DNA repair proteins and trigger a signal to repair the telomere regions by different alternative pathway which in turn could lead to lengthening of the telomere when coupled with mutations in tumor suppressor and oncogenes. The shelterin proteins have an important function to keep the telomerase away from chromosomal ends in adult mammalian cell. When their functioning is disturbed, it causes activation of telomerase to the telomere. That result in lengthening of the telomere under conditions mentioned above. This happens in synchronisation with cancer driving mutations.

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