THE ROLE OF TELOMERIC PROTEINS AND THEIR RELEVANCE AS TARGETS IN ANTI-CANCER THERAPY

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Abstract

The telomere is a region of vital nucleoprotein structures at the terminal end of the chromosome with 500- 3000 repeat sequences of TTAGGG. The shelterin and non-shelterin complexes are telomere associated proteins. These proteins protect chromosome ends from fusion and degradation. The non-shelterin complex of proteins is involved in DNA repair while the shelterin complex of proteins is responsible for regulating telomere length. Telomerase enzyme lengthens telomeres and in immortal cells it is activated. Approximately 90% of human cancers are telomerase positive. The inhibition of telomerase has been considered a safer approach than the use of cytotoxic drugs in anti-cancer therapy. However, the induction of delayed cell growth arrest via telomerase inhibition has been shown to be slow. This is due to the fact that the replication rounds required are too many for it to be effective. Induction of dissociation of shelterin proteins from telomeric DNA has been considered a more efficacious approach based on gene silencing and mutation studies.

The loss of shelterin proteins such as the protection of telomeres 1 (POT 1) and telomere repeat binding factor 2 (TRF 2) in cancer cells has been associated with apoptosis of the tumour cells. Poly ADP ribose polymerase (PARP), a non-shelterin protein, is involved in DNA repair. Drugs such as PJ-34 and 3-Aminobenzamide have been developed as PARP inhibitors. Drugs that dissociate telomeric proteins from telomeric DNA are still under clinical trials, they include Quarfloxin, C-1305 and Telomestatin. These drugs have low cytotoxicity and are effective against tumours linked with dysfunction of DNA repair. The level of cytotoxicity of a drug is vital in determining its efficacy. The cytotoxicity of cell-cycle inhibitors as anti-cancer medication motivated researchers to investigate telomeric proteins as targets of anti-cancer intervention. The dissociation of shelterin proteins from telomeric DNA and inhibition of non-shelterin proteins such as PARP and Tankyrase 1 and 2 have shown promising efficacy levels as anti-cancer therapy.

1. Introduction

The telomere is a region of essential nucleoprotein structures made up of DNA-protein complexes with 500-3000 repeats of TTAGGG at the ends of eukaryotic chromosomes (ARTANDI and RONALD, 2010). It is associated with the shelterin and non-shelterin complex of proteins (HOCKEMEYER et al., 2005). These complexes regulate telomere length and repair damaged DNA respectively. The shelterin is a complex of six known sub-units that safeguard the telomere from detection by DNA damage response factors and regulates its length (DE LANGE, 2005). The non-shelterin complex is structurally part of the telomeric repeat sequence of TTAGGG that is primarily involved in DNA repair (PALM and TITIA, 2008).

These complexes protect against events that promote genome instability (AZZALIN et al., 2007). These events include the degradation of terminal ends of chromosomes and fusion of telomeres with broken DNA ends (BLACKBURN, 2005; CECH, 2004; WANG et al., 2007). The major consequence of fusion of telomeres is the formation of dicentric chromosomes (BLASCO et al., 2005). These dicentric chromosomes are unbalanced and may result in uneven distribution of the genetic content of dividing cells or loss of genetic information (DE LANGE, 2009). In cancerous cells, dicentric chromosomes and fused sister chromatids are correlated with critically shortened telomeres (O' SULLIVAN and KARLSEDER, 2010).

The shortening of telomeres is associated with chromosome instability (AZZALIN et al., 2007). In somatic cells whose telomeres undergo shortening with age, telomerase has been shown to be inactive (ZAKIAN, 1995). In immortal cells, this shortening is arrested as a consequence of their ability to express telomerase. Multiple studies have shown that telomerase is active in 90% of cancer cells (SMOGORZEWSKA and TITIA, 2004; VERDUN and KARLSEDER, 2007; WANG et al., 2007).

The reactivation of telomerase in murine models has been shown to aggravate prostate cancer (CHAN and BLACKBURN, 2003). The telomeres of germline and cancer cells do not shorten. The protection of telomere 1 (POT 1) is an example of the shelterin complex of proteins (COLGIN et al., 2003). Its main function is to regulate telomere lengthening. Its mutation has been linked to chronic lymphocytic leukaemia (RAMSAY et al., 2013). Telomeric repeat binding factor 1 (TRF 1), also a shelterin complex protein has been linked with the regulation of telomere length (MAKAROV, YOKO and LANGMORE, 1997). Its mutation has been linked with adenocarcinoma of the lung (NAKANISHI et al., 2003). The dissociation of POT 1 and

TRF 1 from the telomeric DNA in the aforementioned cancers culminated in a near remission of the malignancies using drug candidates such as Quarfloxin (NEIDLE and PARKINSON, 2002). This stimulates release of DNA damage response factors such as ATM protein culminating in apoptosis.

Apoptosis of the tumor cells can also be induced by another drug C-1305 which is still under trial. C-1305 is a triazoloacridone derivative that binds to the G rich strand of the TTAGGG repeat sequence of telomeres (NEIDLE, 2010). Telomestatin has also shown promise in the induction of TRF 2 dissociation (KIM et al., 2002). The exact mechanism of action of these drugs is yet to be elucidated. The direct inhibition of non-shelterin proteins has also shown promise in anti-cancer therapy. PARP and Tankyrase 1 and 2 have been investigated as anti-cancer targets. PARP inhibitors such as PJ-34 and 3-aminobenzamide have low cytotoxicity and are efficacious in inducing apoptosis (ARTANDI and RONALD, 2010). These drugs have a low cytotoxicity as compared to the current anti-cancer medication. There are numerous studies that have been done on the telomeric proteins in an attempt to elucidate their structure and functions. Currently, drugs are being developed that target the telomeric proteins as an anti-cancer intervention. This review attempts to integrate the information on the functions of telomeric proteins and on current anti-cancer drugs that are targeting specific telomeric proteins.

2 Materials and methods

Preliminary searches were conducted using the search engines Google and PubMed, with keywords being shelterin and non-shelterin complex of proteins. Once sufficient literature had been obtained with these keywords, the same search engines were employed using the keywords telomeres and anti-cancer therapy. Subsequently, articles were obtained from relevant journals and journals which, either entirely or by specific issues or volumes, had specific articles on protection of chromosomes by telomeres and telomerase. Any additional relevant studies from the reference lists of these papers were also included. Only human and animal studies published after 1995 in English language were included. Letters, editorials and practice guidelines were excluded. Articles with restricted access were excluded from the review.

Literature Review

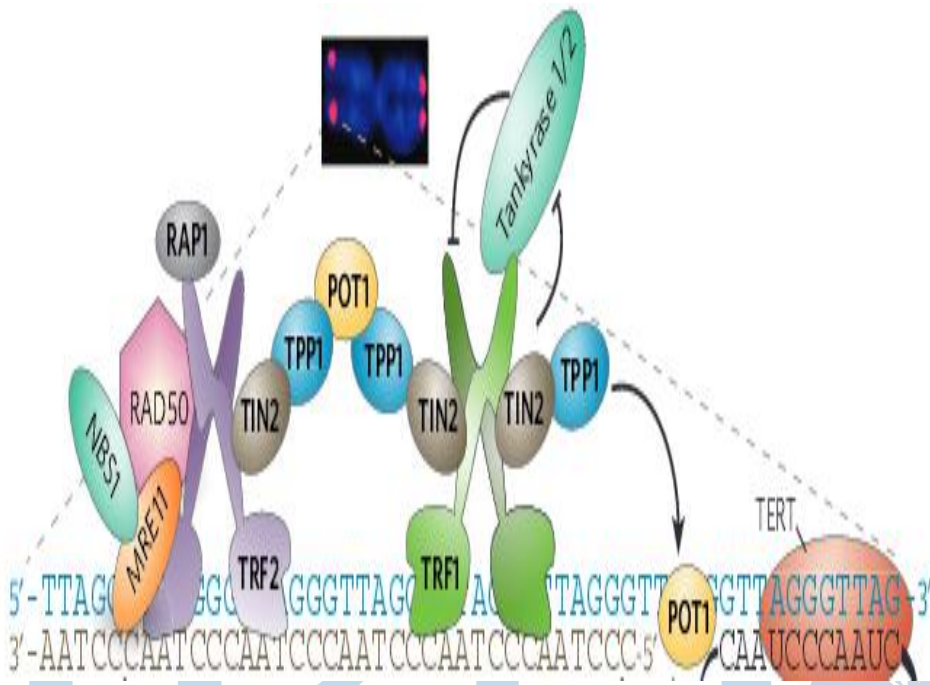
The telomere is a region of the terminal end of the eukaryotic chromosome with a 500-3000 repeats of TTAGGG. It is associated with the shelterin and non-shelterin complex of proteins.

Deciphering the role of shelterin and non-shelterin proteins in malignant transformations may be the new bastion of hope in the battle against tumorigenesis. The link may aid in providing a targeted approach in therapeutic interventions.

- **Role of Shelterin complex**

The telomere is a region of vital nucleoprotein structures made up of tightly regulated DNA-protein complexes (SHAY and WOODRING, 2005). It is made up of approximately 150 nucleotides with 500-3000 repeats of TTAGGG at the ends of eukaryotic chromosomes (ARTANDI and RONALD, 2010). The size of the telomere ranges from approximately 15 kb at birth to <5 kb in chronic diseases in adults (SHAY and WOODRING, 2005). The principal role of telomeres is to conceal the ends of linear eukaryotic chromosomes from detection by DNA damage response factors and thus prevents improper repair (VERDUN and KARLSEDER, 2007). It is associated with the shelterin and non-shelterin complex of proteins (DE LANGE, 2005).The shelterin complex of six proteins (Fig. 1) which include: telomeric repeat binding factor 1 (TRF 1), telomeric repeat binding factor 2 (TRF 2), TRF 1 Interacting protein 2 (TIN 2), protection of telomeres 1 (POT 1), and tripeptidyl-peptidase 1 (TPP 1). TRF 1 and 2 control telomere length (DE LANGE, 2005).

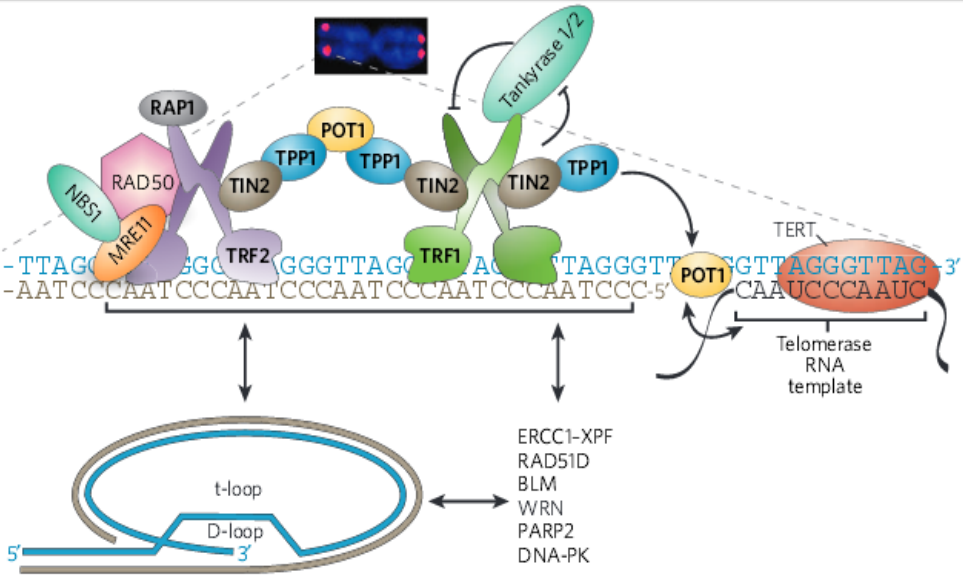
Figure 1: The proteins forming the shelterin complex (Adapted from Titia De Lange, 2005)



The TRF 1 protein is present at telomeres throughout the cell cycle. It is a negative regulator of telomerase by acting in cis to limit the elongation of telomere (BROCCOLI et al., 1997). Telomere length is also regulated by TRF 2 which inhibits end to end fusion of the telomere by repressing the ataxia telangiectasia mutated pathway of DNA damage response (BILAUD et al., 1997). TRF 1 and 2 are linked and stabilized by the TIN 2 protein as shown in figure 1 above. It has been postulated that TRF 1 is inadequate for maintenance of telomere length and that the TIN 2 protein is a mediator of its functions (YE and TITIA, 2004). The inhibition or knockdown of TIN 2 has been shown to cause telomere elongation this can be explained by the fact that it is a mediator of TRF 1 functions which include telomerase inhibiton. Thus, inhibition of TIN 2 results in diminished activity of TRF 1 and consequently telomerase activity increases (YE and TITIA, 2004). TIN 2 has also been shown to protect TRF 1 from the poly ADP ribosylation effect of a non-shelterin protein known as tankyrase 1. The ADP ribosylation reduces the activity of TRF1 thus increasing telomerase activity (KIM et al., 2008). In studies where TIN 2 has been inhibited by small interfering RNA (si RNA) and the anti-tankyrase 1 function lost, tankyrase inhibiting drugs such as 3 aminobenzamide have been used to rejuvenate the activity of TRF 1 and inhibit telomerase (NEIDLE, 2010).Telomerase activity is dependent on the integrity of the

telomeric DNA. POT 1 has been shown to protect this integrity by inhibition of DNA damage surveillance mechanisms such as the ataxia telangiectasia mutated pathway (YANG, ZHENG and CURTIS, 2005). Shelterin proteins have some common features which include: being highly concentrated only at the chromosomal ends, maintaining integrity throughout the cell cycle and their functions are only limited to the telomeres (NEIDLE and PARKINSON, 2003). Shelterin plays a key role in the formation of t-loops and controlling synthesis of telomeric DNA, TRF 2 has been shown to be responsible for promoting formation of t-loops (DE LANGE, 2005). T-loops are a nucleosomal organization of duplex strands that masks chromosome ends from being detected as broken DNA thus prevents release of DNA damage response factors (SHORE, 2001). They protect chromosome ends from non-homologous end joining and degradation (MASUTOMI et al., 2003). The t-loop protects the 3' overhang (Fig. 2). The 3'overhang is a single stranded 3' protrusion of a G-rich strand. It is also known as a G-tail or G-overhang (PAESCHKE et al., 2005)

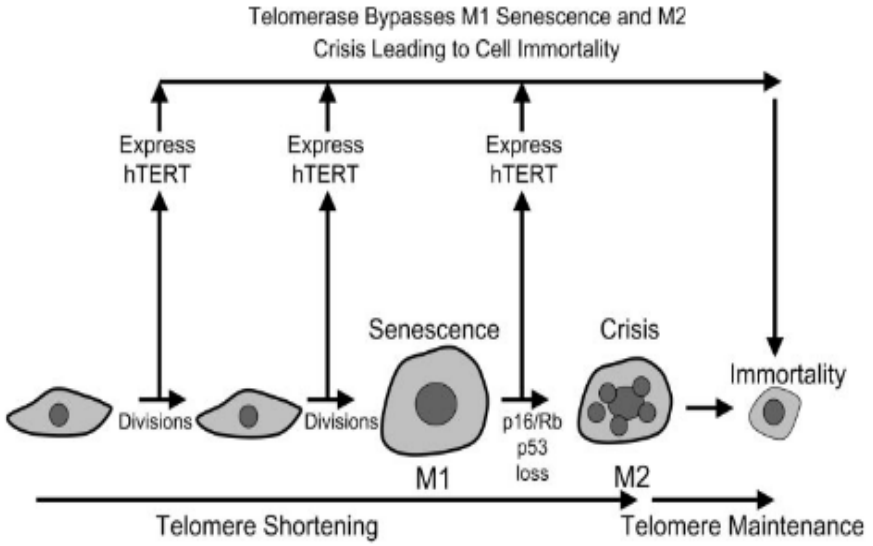
Figure 2: T- loop formation and the 3' overhang (Adapted from Titia De Lange, 2005)



The 3' overhang could be lost in certain events such as the resolution of the t-loop due to progressive telomere shortening (MITTON-FRY et al., 2002). The loss of 3'-G overhang results in growth arrest or apoptosis (REICHENBACH et al., 2003).Inhibition of TRF 2 also causes loss of 3' overhang. TRF 2 is a minute dimeric protein which attaches on the hexameric (TTAGGG) repeat sequence. Its dysfunction results in non-homologous end associations (recombination) of

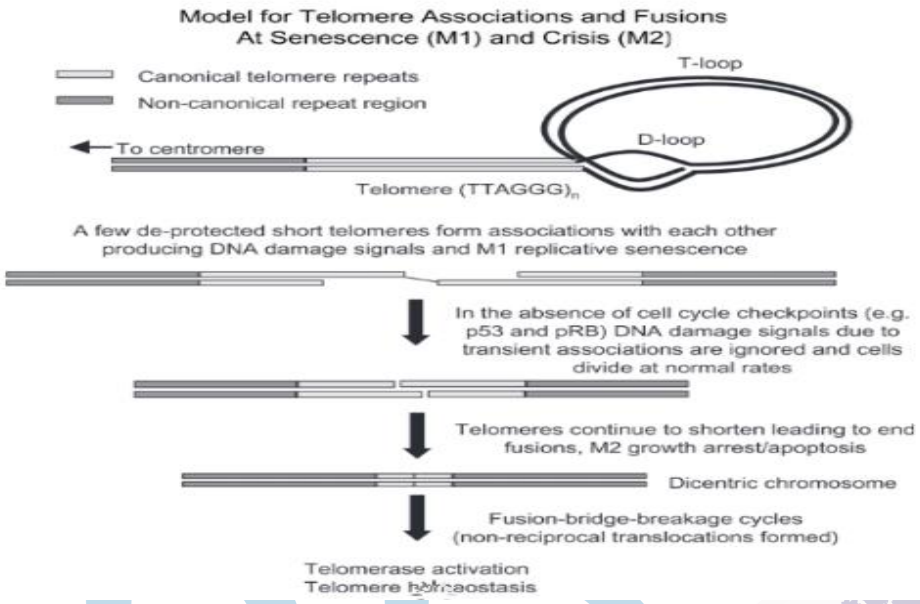
chromosomes (HAHN, 2003). The formation of end associations occurs when only a few telomeres are short. This results in release of DNA damage response signals culminating in replicative senescence (VERDUN AND KARLSEDER, 2006). In replicative senescence, there is silencing of the genes near telomeres. Replicative senescence has two stages, M1 and M2 (AZZALIN et al., 2007). M1 stage involves cellular growth arrest while M2 is marked by apoptosis (Fig.3).

Figure 3: Replicative senescence, M1 and M2 stages.



The major features of cellular growth arrest include; inability of cells to divide even when stimulated by mitogens, maintenance of metabolic activity and morphological changes of the cell (O' SULLIVAN and KARLSEDER, 2010). The continuous shortening of telomeres at M1 stage results in the M2 (crisis stage) which has unique features (AZZALIN et al., 2007). These features include; numerous apoptotic cells, depleted telomere proteins at the chromosomal ends (uncapped) and chromosomal end fusions (TAKAKURA et al., 2013). A number of cells have mechanisms to bypass both M1 and M2 stages. M1 is bypassed when the cell cycle regulators are inhibited, dysfunctional or absent (TAKAKURA et al., 2013). The absence of P53 allows cells to circumvent M1 senescence (Fig. 4).

Figure 4: Telomere associations and fusions at senescence (M1) and crisis (M2)



M2 is bypassed by reactivation or up-regulation of the telomerase enzyme; this occurs in approximately 1 in 10 million human cells (VERDUN AND KARLSEDER, 2006). The bypass of M2 stage by reactivation of telomerase activity is responsible for immortalization of cells and this leads to cancer.

- **Role of non Shelterin complex**

The non-shelterin complex of proteins is made up of several proteins (RHYU, 1995). Some of the proteins are RNA polymerase factors e.g. Xeroderma Pigmentosum group F which removes nonhomologous 3' tail ends during homologous recombination (WRIGHT et al., 1997). Its mutation leads to the condition known as Xeroderma Pigmentosum which predisposes patients to squamous cell carcinoma and metastatic malignant melanoma (BLACKBURN, 2000).

Tankyrase 1 protein is involved in the post translational modification of proteins which maintain telomere length and associate sister telomeres (SEIMIYA and SMITH, 2002). Tankyrase 1 is involved in poly ADP ribosylation of TRF 1 which diminishes its functions; however, this function is mitigated by the TIN 2 protein. Tankyrase 1 is a target of anti-cancer medication (SEIMIYA and SMITH, 2002). 3 aminobenzamide is a tankyrase inhibitor which restores TRF 1 functions which include telomerase inhibition. Other poly ADP ribosylation proteins are principally involved in DNA repair and apoptosis via caspase activity (HAHN, 2003).

The repair of damaged DNA is a critical event in prevention of malignant transformation (VERDUN and KARLSEDER, 2007). The excision repair cross-complementing 1 (ERCC 1) is an nsp involved in nucleotide excision repair of damaged DNA (PAESCHKE et al., 2005). Xeroderma Pigmentosum group F has been shown to act synergistically with ERCC 1 to cut the 5' end of damaged DNA.

In patients with non-small cell lung carcinoma, ERCC 1 positivity is a favourable prognostic marker (YANG, ZHENG and CURTIS, 2005). Their chance of overall survival is increased by the expression of ERCC1. The meiotic recombination 11 homologue (MRE 11) protein also has prognostic value in cancerous conditions (HWANG et al., 2008). It's useful in the prediction of treatment outcome in bladder cancer. It has 3'-5' exonuclease activity and is involved in DNA double strand break repair. The 3'-5' exonuclease activity is commensurate with the action of DNA helicases that unwind simple, partial duplex DNA substrates with 3'-5' polarity. These DNA helicases include the Bloom's syndrome protein and Werner's syndrome protein (VAN STEENSEL, SMOGORZEWSKA and TITIA, 1998).

A mutation in the Werner's syndrome protein increases the risk of acquiring cancers such as malignant melanoma, soft tissues sarcoma and malignant fibrous histiocytoma (ZHU et al., 2003). The recently developed drug, SB203580, inhibits the p38 signaling pathway which is activated by the mutation of Werner's syndrome protein (DAVIS et al., 2005). The p38 pathway is active during genomic instability and has been shown to contribute to premature aging.

Conversely, the p53 pathway is activated via phosphorylation by the ataxia telangiectasia mutated (ATM) protein culminating in either cell cycle arrest or DNA repair (VERDUN and KARLSEDER, 2007). ATM also phosphorylates another tumor suppressor, H2AX. This allows the nucleosome to be less condensed thus allowing space for DNA repair factors to attach after a double strand break (AZZALIN et al., 2007). A defect in the ATM protein has been associated with: atypical B cell chronic lymphocytic leukemia, adult T-cell leukemia, and mantle cell lymphoma (YANG, ZHENG and CURTIS, 2005).

- **Shelterin and non-shelterin proteins as targets in anti-cancer therapy**

The dissociation of shelterin proteins from telomeric DNA in cancerous cells has proven effective in growth arrest of the cells (GREIDER, 1996). The mechanism involves disruption of the 3' overhang by conversion to a quadruplex folded state by using drug candidates such as Quarfloxin (NEIDLE and PARKINSON, 2002). The drug is still under clinical trials and its role in 3' overhang disruption prevents POT 1 from binding to the telomeric DNA (ZAUG, PODELL and CECH, 2005). This stimulates release of DNA damage response factors such as ATM protein culminating in apoptosis.

Apoptosis of the tumor cells can also be induced by another drug C-1305 which is still under trial. C-1305 is a triazoloacridone derivative that binds to the G rich strand of the TTAGGG repeat sequence of telomeres (NEIDLE, 2010). It has been shown to cause unbinding of TRF 1 and 2 which results in replicative senescence. TRF 2 dissociation is accompanied by anaphase bridge formation leading to 3' overhang loss which consequently results in apoptosis (KIM et al., 2002). Telomestatin has also shown promise in the induction of TRF 2 dissociation (KIM et al., 2008). The exact mechanism of action of these drugs is yet to be elucidated.

It is postulated that the TPP1 glutamate (E) and leucine (L) patch aka TEL patch on the shelterin protein TPP 1 recruits the enzyme telomerase to chromosome ends. Inhibition of the binding of telomerase enzyme to the TEL patch of TPP 1 has been shown to cause reduction of telomere elongation (NAKANISHI et al., 2003). This inhibition is believed to be one of the mechanisms that the telomere specific drugs utilize.

The direct inhibition of non-shelterin proteins has also shown promise in anti-cancer therapy. PARP and Tankyrase 1 and 2 have been investigated as anti-cancer targets. The PARP inhibitors allow TRF 1 to inhibit telomerase. These inhibitors such as PJ-34 and 3-aminobenzamide have low cytotoxicity and are efficacious in inducing apoptosis (ARTANDI and RONALD, 2010). However, expression of some of the non-shelterin proteins such as ERCC 1 reduces the efficacy of platinum based therapeutic interventions (HWANG et al., 2008). The mutations of non-shelterin proteins are associated with cancers. The Werner's syndrome protein mutation is associated with predisposition to cancers such as sarcoma and melanoma. The drug SB203580 is involved in the inhibition of the p38 pathway (DAVIS et al., 2005). This pathway may be involved in the process of cellular aging. The targeting of

the pathway may be more beneficial in preventing premature aging than delaying the malignant transformation of cells (PAESCHKE et al., 2005).

Conclusion

The level of cytotoxicity of the current anti-cancer drugs has led to the search for less toxic drugs. The search has led to the development telomerase inhibiting drugs. Telomerase is active in germline and tumor cells. It interacts with telomeric DNA to cause elongation of telomeres. The telomeres are made up of shelterin and non-shelterin proteins. Shelterin proteins regulate telomere length. The dissociation of shelterin proteins, TRF 2 and POT 1, from telomeric DNA by drugs such as Quarfloxin, C-1305 and Telomestatin results in apoptosis. The non-shelterin proteins repair DNA. The inhibition of these proteins by drugs such as PJ-34 and 3-aminobenzamide has shown promise in the induction of apoptosis. The mechanism of action of these drugs is yet to be elucidated. The success of these drugs in current clinical trials has provided a new bastion of hope in the war against cancer. Further research into mechanisms of dissociation of other shelterin proteins and inhibition of appropriate non-shelterin proteins is required to augment the milestones achieved.

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