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Telomeres and telomerase in prostate cancer development and therapy

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Abstract

Aberrations in telomere biology are among the earliest events in prostate cancer tumorigenesis and continue during tumour progression. Substantial telomere shortening occurs in prostate cancer cells and high-grade prostatic intraepithelial neoplasia. Not all mechanisms of telomere shortening are understood, but oxidative stress from local inflammation might accelerate prostatic telomere loss. Critically short telomeres can drive the accumulation of tumour-promoting genomic alterations; however, continued telomere erosion is unsustainable and must be mitigated to ensure cancer cell survival and unlimited replication potential. Prostate cancers predominantly maintain telomeres by activating telomerase, but alternative mechanisms of telomere extension can occur in metastatic disease. Telomerase activity and telomere length assessment might be useful in prostate cancer diagnosis and prognosis. Telomere shortening in normal stromal cells has been associated with prostate cancer, whereas variable telomere lengths in prostate cancer cells and telomere shortening in cancer-associated stromal cells correlated with lethal disease. Single-agent telomerase-targeted treatments for solid cancers were ineffective in clinical trials but have not been investigated in prostate cancer and might be useful in combination with established regimens. Telomere-directed strategies have not been explored as extensively. Telomere deprotection strategies have the advantage of being effective in both telomerase-dependent and telomerase-independent cancers. Disruption of androgen receptor function in prostate cancer cells results in telomere dysfunction, indicating telomeres and telomerase as potential therapeutic targets in prostate cancer.

Early studies of linear yeast artificial chromosomes identified three essential elements required to ensure correct duplication and segregation of linear eukaryotic chromosomes[1]: an origin of replication[2,3], a centromere[4,5], and a pair of telomeres at the extreme ends of the chromosome[6]. Human telomeres consist of a highly conserved, G-rich, repetitive hexanucleotide sequence $(TTAGGG)_n$[7,8], approximately 5–15 kb in length[9]. A complex of six proteins — collectively termed shelterin — is associated with the telomeric DNA repeats (telomeric repeat-binding factor 1 and 2 (TERF1 and TERF2, also known as TRF1 and

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TRF2), TRF2-interacting protein 1 (TERF2IP, also known as RAP1), TRF1-interacting nuclear factor 2 (TINF2, also known as TIN2), protection of telomeres protein 1 (POT1), and tripeptidyl-peptidase 1 (TPP1))[10–20]. The linear nature of human chromosomes poses several biological dilemmas that these telomeric nucleoproteins help mitigate. The extreme ends of chromosomes are potential substrates for exonucleolytic degradation and can also be recognized as a DNA double-strand break by the DNA damage response (DDR) pathway. Telomeres of sufficient length safeguard against exonuclease activity and DDR recognition by forming specialized T-loop structures[21] and serve as a scaffold for shelterin proteins that inhibit potentially deleterious DNA repair mechanisms at the telomeres[20].

Telomeres are integral to cellular proliferation barriers that ensure finite replicative capacity in cells, serving as a potent anticancer mechanism[22,23]. During DNA replication, synthesis on the lagging DNA strand of linear templates is incomplete (end replication problem)[24,25], resulting in the loss of ~50 terminal nucleotides in each round of cellular division[26]. For a limited number of population doublings, telomeres buffer against the loss of information-carrying DNA sequences. However, when telomeres become substantially shortened, the DDR pathway is activated on one or more telomeres, and cell cycle progression is arrested via the tumour suppressor p53 pathway[27–29]. This state of cell cycle arrest is termed replicative senescence and occurs after ~50 cell divisions (the Hayflick limit), depending on cell type. Failure to block cell cycling via the p53 pathway can have devastating genomic consequences, manifested as end-to-end chromosomal fusions, anaphase bridges, nonreciprocal translocations and aneuploidy. All these processes help to promote cellular transformation via the stochastic inactivation of tumour suppressor genes and activation of oncogenes[30–34].

To achieve unlimited replicative capacity, cancer cells must eventually resolve the end replication problem. Predominately, cancers maintain their telomeres by activating telomerase, a telomere-specific enzyme that extends telomeres[35], therefore obviating the end replication problem. Approximately 5–10% of cancers employ a telomerase-independent mechanism to maintain and extend telomeres called the alternative lengthening of telomeres (ALT), which is thought to rely on homology-directed DNA recombination[36,37]. ALT is frequently observed in nonepithelial cancers, but ALT has been reported in a subset of advanced, lethal metastatic prostate tumours[38] (FIG. 1). By contrast, ALT has not been observed in primary prostate cancers, of which the vast majority, if not all, employ telomerase for telomere maintenance[39].

In this Review, we highlight the function of telomeres that specifically relate to prostate cancer. We describe the short-telomeres phenotype observed in the majority of precancerous prostate lesions and prostate tumours and the potential sources of telomere shortening in prostate cancer. Furthermore, we discuss how telomere shortening and telomerase activation result in genomic instability, consequently contributing to tumour promoting mutations. Finally, we delineate the clinical implications of telomere dysfunction and the therapeutic potential of treatments targeting telomerase and telomeres in patients with prostate cancer.

Role in prostate pathophysiology

Telomerase, a reverse transcriptase, extends chromosome ends with a concatemer of the telomeric TTAGGG repeat sequence[40]. The creation of the enzyme is a complex orchestration of several proteins and nucleic acids participating in the biogenesis and localization of telomerase[41]. The core components for DNA extension activity consist primarily of the catalytic telomerase reverse transcriptase protein (TERT) and the telomerase RNA component (TERC, also known as hTR), which contains the repetitive telomeric sequence that functions as a template for the reverse transcriptase[42,43]. Telomerase expression is repressed in most human somatic cells, but detectable levels of activity exist in germline and somatic stem cells[44].

Prostate cancers activate telomerase to maintain telomeres, but the telomeres in prostate cancer cells, as directly assessed *in situ*, are abnormally short in the vast majority of clinical samples compared with matched adjacent normal prostate tissue[39]. Southern blot analysis of terminal restriction fragments (TRFs) shows that the average telomere length of normal prostate tissue is 6.6 kb, whereas prostate cancer tissue has a considerably shorter average telomere length of 5.4 kb[39]. This finding is perhaps not surprising, considering that even adult stem cells in epithelial tissues with activated telomerase are subject to telomere shortening over time, presumably because the amount of telomerase present is unable to fully offset the end replication problem. In the same study, the average telomere length of BPH tissue was 6.4 kb, which is comparable to that of the normal prostate. These reported telomere lengths are probably an overestimation of the actual telomere length, as the TRF Southern blot technique detects not only pure TTAGGG telomeric repeats but also degenerate and variant subtelomeric sequences, resulting in estimates that are 4 kb higher than the actual telomere region consisting exclusively of telomere repeats[26,45]. In addition, contaminating normal cells artificially increase measured average telomere lengths in bulk tissue analyses.

Similar to prostate cancer, prostatic intraepithelial neoplasia (PIN) and BPH are characterized by an abnormal increase in cell proliferation. Localized prostate cancer has an ~7-fold increased cell proliferation rate compared with normal prostatic epithelial tissue[46]. High-grade PIN (HGPIN) tissue proliferates ≥6-fold faster than normal prostate[46], and has abnormally short telomeres[47]. BPH tissue proliferates at a 2–3-fold higher rate than normal prostate[48] but, surprisingly, does not display the telomere shortening expected to accompany abnormally high cell proliferation in a telomerase-negative setting[39].

Kinetic studies of abnormal lesions in the prostate also show that BPH tissue is indeed less proliferative than PIN tissue[49], which might partially account for the lack of telomere shortening observed in BPH. Alternatively, the inconsistency in telomere length might be caused by a difference in the proliferative topology between BPH and HGPIN tissues[50]. Normally, proliferation in prostatic epithelial tissues is mainly restricted to the basal cell compartment, where stem cells are thought to reside[51–53]. However, in HGPIN lesions, hyperproliferation occurs in both basal and luminal epithelial cells[54,55] and the majority of proliferation occurs in the luminal compartment[50,53,56]. Correspondingly, telomere shortening in HGPIN tissue is observed in luminal cells only and not the basal cell

compartment[47]. By contrast, hyperproliferation in BPH occurs in both stromal and epithelial cells. Furthermore, proliferation of epithelial cells in BPH mainly occurs in the basal compartment[53,56], similar to normal prostate, which implicates stem cells and stem-like cells in the aetiology of BPH. Consistent with this notion, a report from 2016 suggested that the telomerase-negative epithelial cells in BPH with normal telomere length arise from telomerase-positive progenitor cells that differentiated from stem-like cells residing in the basal compartment[57]. Thus, the lack of short telomeres in BPH could be caused by widespread proliferation of multiple progenitor cells, in contrast to cancer, which is traditionally thought to be the result of clonal expansion originating from a single transformed cell. HGPIN lesions share many features with prostate cancer; hence, HGPIN are believed to be the malignant precursors of prostate cancer[58,59], which strongly implicates telomere shortening as an early event in prostate tumorigenesis (FIG. 1). Evidence to define a concrete timeline of telomere shortening and the transition point from HGPIN to cancer is limited, but telomerase activation is likely to be a key event. The precise timing of telomerase activation during prostate tumorigenesis is unknown. However, studies have indicated that at least a subset of HGPIN lesions have detectable telomerase[60,61], suggesting that considerable telomere shortening ostensibly occurs before telomerase activation in HGPIN.

Cancer is a disease of ageing, and prostate cancer is not unusual in that regard. The median age for prostate cancer diagnosis is 67 years[62]. Prostate cancer is an exceptionally slow-growing cancer compared with other solid tumours[63]. A clonal outgrowth of prostate cancer has been estimated to take ~40 years to reach a size of 1 cm^3 (REF. 46). The even lower rate of proliferation in the normal prostate is not consistent with a timeline that would result in substantial telomere shortening before transformation, and such substantial shortening is also not observed. On the basis of an estimated 500-day turnover rate of the prostatic epithelium[46], and the Hayflick limit of 50 population doublings[23,64], normal prostate cells would take ~68 years to reach replicative senescence. Furthermore, prostate cells would take an additional 27 years of proliferation to achieve critical telomere lengths, when one or more telomeres become dysfunctional. These estimates assume that, during tumorigenesis, prostate cells need to bypass replicative senescence, that 50 bp of telomere content are lost per division[26], and that the difference between senescent cells and cells in crisis is 1 kb of telomere content[65]. On the basis of these conservative numbers, prostate cells would need to proliferate for almost 100 years before telomeres were sufficiently shortened to result in the telomere-driven genomic instability required for normal prostate cells to develop into precursor lesions. Taken together, the combined observations of relatively slow proliferation rates of normal prostate cells and luminal-cell-specific telomere shortening in prostate cancer and precursor HGPIN lesions suggest that additional factors (for example, oxidative stress, perhaps from local inflammatory processes) might work in concert with cell proliferation to accelerate the process of telomere shortening in precancerous HGPIN lesions and perhaps further in prostate cancer.

Telomeres and genomic alterations

Oxidative DNA damage and inflammation

Telomeres are particularly susceptible to telomere shortening in response to DNA damage, which might be aggravated by persistent inflammation and the production of reactive oxygen species (ROS) (FIG. 2). ROS are inherent byproducts of cellular respiration, arising from multiple endogenous sources, including the electron transport chain in mitochondria[66]. However, additional endogenous and exogenous sources of free radicals can increase ROS levels above baseline with devastating cellular consequences. A particularly well-characterized effect of elevated ROS levels is increased DNA damage[67]. The predominant species of free radicals that contribute to cellular DNA damage are hydroxyl radicals (·OH) arising from superoxide radicals ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2)[68–70]. Guanine has the lowest oxidation potential of the DNA bases[71]. As a result, the oxidation of guanine to form 8-oxoguanine is the most abundant of the many DNA mutations that arise from ROS exposure[72].

Telomere sequences are rich in guanine nucleotides and, consequently, telomeres are particularly susceptible to oxidative damage by ROS. Cells in culture exposed to increasing amounts of radiation have a dose-dependent increase in 8-oxoguanine abundance and telomere loss[73]. 8-Oxoguanine DNA lesions are recognized and excised by 8-oxoguanine glycosylase and the abasic site is repaired via the base excision repair (BER) pathway[74]. During BER, a single-strand DNA break is generated following the glycosylase step by AP endonuclease 1. BER operates during all phases of the cell cycle, including in dividing cells[75], which is particularly relevant, as single-strand DNA breaks in telomeres seem to accelerate the rate of telomere shortening in proliferating cells[76]. Telomeres have a higher frequency of single-strand DNA breaks than any other part of the genome, single-strand breaks induced by oxidative damage in telomeres accumulate and repair is substantially delayed[77]. The reduced repair efficiency in telomeres[78] might in part be explained by suppression of DNA repair by the shelterin complex to prevent telomere ends from being recognized as double-strand breaks[79]. In addition to oxidative damage, the abundance of guanine nucleotides in telomeres also makes them susceptible to the formation of G-quadruplexes, which interfere with replication[80]. The helicases BLM and RTEL1 resolve G-quadruplexes at telomeres and, in cooperation with the shelterin protein TRF1, help prevent replication fork stalling to ensure efficient replication at telomeres[81]. However, these processes are not perfect and telomeres are prone to develop DNA strand breaks; thus, they behave as fragile sites in the chromosome[81]. One hypothesis is that DNA damage, such as that caused by oxidative stress, exacerbates the fragile site phenotype of telomeres and that incomplete replication at telomeric ends caused by replication fork stalling at persistent sites of DNA lesions promotes critical telomere shortening[82]. In addition to metabolic sources of free radicals, inflammation can locally increase levels of ROS in tissues. ROS are generated by immune cells as part of the immune response against infection by pathogens, acting as signalling molecules, nonspecific antimicrobials, and mediators of inflammation[83].

Inflammation is an important component in the initiation and progression of many cancers and accumulating evidence supports a similar role in prostate cancer[84–86]. The presence of

inflammation, mostly chronic, in benign prostate biopsy tissues was found to be associated with prostate cancer, particularly high-grade disease[87]. Furthermore, use of anti-inflammatory medications, specifically acetylsalicylic acid and other nonsteroidal anti-inflammatory drugs, is associated with reduced risk of developing prostate cancer[88]. Prostatic inflammation is common[86,89–93] and inflammatory triggers include infectious agents (for example, bacteria and viruses), dietary factors, oestrogen exposure, physical trauma from corpora amylacea, and the physical and chemical irritation from urine reflux[85,94,95].

The histological sequelae, presumably caused by acute and/or chronic inflammation in the prostate, include prostatic inflammatory atrophy (PIA)[85]. These morphologically atrophic lesions are characterized by an increased proliferation of epithelial cells (to a mean proliferation rate of 10.7-fold above normal epithelial cells)[96–98] and the presence of activated inflammatory cells (predominately lymphocytes and macrophages)[96,99]. These proliferation rates would lead epithelial cells in PIA lesions to have increased telomere shortening and, therefore, to reach replicative senescence considerably faster than normal prostate cells. Thus, PIA has been suggested to be a precursor to PIN and subsequent prostate cancer[85]. Intriguingly, PIA lesions tend to histologically merge into HGPIN in the prostate[100], supporting the notion that PIA is a precursor to PIN. In addition, the relative frequency of prostatic inflammation is similar to the demographic frequency of prostate cancer in men. For example, American men of African origin are at increased risk of developing prostate cancer[101] and have increased rates of prostatic inflammation compared with American men of European decent[102]. One interesting observation of prostate cancer epidemiological data is that American men with parents who have migrated from Asia have substantially increased rates of prostate cancer compared with Asian men who did not migrate, strongly implicating an environmental component[103]. Switching from an Asian, possibly anti-inflammatory diet including tea and soy products to a Western, pro-inflammatory diet has been suggested as a contributing factor to the increased prevalence of prostate cancer observed in men of Asian descent[104].

Taken together, the link between telomere shortening and prostate cancer might, in part, be explained by connecting telomere shortening to oxidative stress: the generation of ROS owing to inflammation, the preponderance of inflammation in the prostate, and the association between prostatic inflammation and prostate cancer, potentially driven largely by environmental factors, such as microorganisms or diet. As a precursor to PIN and subsequently prostate cancer, an inflammatory environment potentially drives PIA towards malignant transformation.

Interestingly, in addition to PIN and prostate cancer cells, telomere shortening has also been observed in normal stromal cells and is associated with increased prostate cancer risk[105]. The specific stromal cell types that have short telomeres have yet to be identified, but the telomere shortening in these stromal cells might be a direct result of inflammation.

Inflammation also seems to have a role in the development of BPH[106], but BPH does not display substantial telomere shortening in contrast to HGPIN and prostate cancer[39,47]. Presumably, the telomerase activity in the postulated epithelial progenitor cells and stem-like

cells in BPH would not safeguard against genomic insults from ROS. However, glutathione *S*-transferase P (GST-P), which is expressed in normal prostate and BPH but not in HGPIN or prostate cancer[107,108], does have activity against reactive oxidants and electrophiles that could damage DNA[109,110]. Perhaps, BPH is not susceptible to telomere shortening from hyper-proliferation and oxidative DNA damage because of the maintenance activity of telomerase in postulated epithelial progenitor cells and stem-like cells[57] and the detoxification activity of GST-P[50], respectively. By contrast, the short telomeres in HGPIN should result in considerable selective pressure for halting telomere loss. During tumorigenesis, critically short telomeres can precipitate many important cancer- promoting mutations, such as telomerase activation, by encouraging global genomic instability.

Genomic alterations due to short telomeres

Telomere shortening is one of the earliest molecular genomic events in prostate tumorigenesis and can generate genomic instability. Genomic translocation events are prevalent in prostate cancer and were traditionally thought to accumulate gradually during tumorigenesis. However, chromothripsis can occur, in which multiple translocation events occur in a single catastrophic event leading to imperfect rearrangement and repair of one or a few shattered chromosomes[111–113]. Mutations in the genome resulting from chromothripsis can be involved in tumour initiation or progression through the generation of fusion genes, inactivation of tumour suppressors, and amplification of oncogenes[114–117]. Cell culture experiments with artificially shortened telomeres have demonstrated that critically short telomeres can precipitate chromothripsis[118]. Approximately 30–45% of prostate cancers show DNA rearrangements resembling the translocation events typified by chromothripsis[119].

Chromothripsis notwithstanding, early contributions of telomere shortening to genomic instability are evident in the form of telomere fusions, which have been reported to occur in >50% of assessed prostate cancer precursor lesions derived from radical prostatectomy specimens[120]. These chromosomal end-to-end fusions of dysfunctional short telomeres can elicit the canonical breakage-fusion-bridge (BFB) cycles, in which missegregation of fused chromosomes during mitosis perpetuates a cycle of fused chromosomes improperly breaking and fusing in an error-prone manner[31]. BFB cycles result in substantial chromosomal rearrangements, particularly duplications and deletions, and nonreciprocal translocations[121]. Studies in well-characterized prostate cancer cell lines have shown that short telomeres can drive the complex chromosomal rearrangements characteristic of prostate cancer through BFB cycles, despite telomerase activation[122].

Telomerase activation in prostate cancer

Most cancer cells activate the enzyme telomerase for telomere maintenance to support unlimited replicative capacity and to prevent an intolerable level of genomic instability. In human cancers, the rate-limiting determinant for telomerase activity is thought to be expression of the catalytic protein subunit TERT. Telomerase-positive cultured human cells contain ~1,150 TERC molecules and ~500 TERT molecules per cell[123]. However, estimates of the number of functional, assembled telomerase complexes range from 20 to 240

complexes per cell[123,124], suggesting an excess of unassembled components of telomerase[123]. The transcription of TERT is tightly regulated and experimental evidence indicates that telomerase activity directly corresponds with *TERT* expression[125,126]. Furthermore, in somatic cells, forced expression of *TERT* is sufficient to reactivate telomerase activity[127–129].

The androgen receptor (AR) is essential for stimulating the expression of genes important for the male phenotype, including the development, maintenance, and function of the prostate[130]. The first *in vivo* study of telomerase activity in the prostate of rats showed that normal prostate glands lacked telomerase activity, but involuted prostate glands, following androgen deprivation via castration, had detectable levels of telomerase activity[131]. Reintroduction of androgen in castrated rats stimulated regrowth of the prostate and phenocopy of the precastration prostate in which no telomerase was detected, suggesting that androgen negatively regulates telomerase activity[131]. Similar observations were later made in rhesus monkeys[132].

In normal human prostate epithelial cells, the AR binds to the TERT promoter and, in cooperation with the tumour suppressor p53, directly represses *TERT* expression[133]. By contrast, androgen activates *TERT* expression in the human prostate cancer cell line LNCaP, which has a point mutation in the *AR* gene that is also recurrently observed in prostate cancer[133,134]. Treatment of prostate cancer lines with methaneseleninic acid, which decreases AR protein levels[135], resulted in a concomitant decrease in *TERT* expression in both AR-wild-type (LAPC-4) and AR-mutated (CWR22Rv1, LNCaP, and LNCaP sublines) cell lines, but not in an AR-negative (DU-145) cell line[136]. The decreased *TERT* expression in the AR-positive cell lines was reported to be a consequence of reduced AR occupancy at the *TERT* promoter.

Cancer cells can activate telomerase activity through upregulation of *TERT* expression in several ways (TABLE 1). Two well-documented mechanisms are hypermethylation of the *TERT* promoter, which has been reported in multiple cancer cell lines[137], and activating point mutations in the *TERT* promoter, which have been observed in cancers of the central nervous system, bladder, thyroid, and skin[138,139]. The methylation status of the *TERT* promoter in prostate cancer has yet to be fully investigated; however, *TERT* promoter mutations have not been observed in prostate cancer[138,140]. Instead, the MYC oncogene has been implicated as a contributor to *TERT* overexpression in prostate cancer. Gene expression and immunohistochemical studies have shown overexpression of MYC RNA and protein in prostate cancer compared with BPH and normal prostate tissue[141–146]. Furthermore, *TERT* overexpression and *MYC* overexpression in prostate cancer correlate[147], supporting previous observations that MYC stimulates expression of *TERT*[148]. The *TERT* promoter contains five GC-boxes, a consensus sequence recognized by the transcription factor SP1, and two E-boxes, a consensus sequence recognized by MYC. The transcription factors MYC and SP1 bind to the *TERT* promoter and cooperatively activate the expression of *TERT*[149]. Depending on the cell type, studies have shown that overexpression of either MYC or SP1 protein is sufficient to activate transcription of *TERT*[149].

Increased levels of MYC are one of the earliest somatic molecular alterations observed in prostate cancer precursor lesions. In histologically normal prostatic tissues, MYC expression is localized to the basal compartment. By contrast, in PIA and both low-grade and high-grade PIN, MYC expression abnormally localizes to the luminal compartment[141]. The precise molecular mechanisms underlying MYC activation in prostate cancer precursor lesions are not well understood. Gain of chromosome 8q, which includes *MYC*, is common in PIN, primary and metastatic prostate cancer, and specific amplification of the *MYC* locus is frequently observed in aggressive disease[150,151]. However, amplification of *MYC* as the cause for increased MYC protein levels in PIN is controversial[152].

Clinical relevance

Telomere dysfunction might be a useful target for improved management of patients with prostate cancer (FIG. 3). Potential applications that are directed at telomeres and telomerase could facilitate disease diagnosis and patient prognosis. In addition, treatments that directly target telomerase or depend on its function, as well as agents that exploit shortened telomeres and disrupted DDR, could have clinical utility in the future.

Diagnosis and prognosis of prostate cancer

Early detection is paramount to ensure the best outcomes for men diagnosed with prostate cancer, but currently available screening methods can result in overdiagnosis and overtreatment[153,154]. Detection of telomerase activation could potentially aid in prostate cancer screening, owing to its high cancer specificity. The primary obstacle to telomerase detection is the low abundance of the enzyme. Telomerase-positive human cancer cell lines contain 20–240 functional telomerase complexes per cell[123,124]. The earliest telomerase activity assays required the use of radiolabeled nucleotides to compensate for the relatively low abundance of the protein[155]. To improve assay sensitivity, a PCR-amplification-based approach termed the telomere repeat amplification protocol (TRAP) assay was developed and used successfully to detect telomerase in prostate biopsy samples[156]. In the TRAP assay, telomerase activity is detected by addition of a synthetic telomere end to a cell or tissue lysate. If telomerase is present, the enzyme will recognize and extend the synthetic substrate, and telomerase extension products can be detected by PCR amplification.

Currently, no telomerase assays for cancer detection are clinically validated. However, considerable effort has been made to incorporate technological advances in signal amplification, nanotechnology, and optical detection to improve the reliability and sensitivity of the TRAP assay[157]. The detection of circulating tumour cells (CTCs) in the blood is a noninvasive procedure that has potential utility in the clinic. Telomerase activity measurements could identify CTCs isolated from the blood of patients with prostate cancer. An ELISA-based TRAP assay was able to identify CTCs in 79% of patients with localized prostate cancer and in 0% of healthy men with no evidence of prostate cancer[158]. A subsequent study using a different version of the TRAP assay demonstrated sensitive and reliable detection of prostate cancer cells in blood collected on a microfilter platform[159]. Further studies evaluated the utility of telomerase detection in live CTCs as a prognostic tool for overall survival in patients with advanced castration-resistant prostate cancer. In a subset

of patients with a baseline count of ≥5 CTCs per 7.5 ml of blood, increased telomerase activity was associated with worse outcomes[160].

Alternatively, telomere length can potentially aid in prostate cancer diagnosis. A study using quantitative telomere-specific fluorescence in situ hybridization (FISH) in biopsy samples from men participating in the placebo arm of the Prostate Cancer Prevention Trial showed that telomere shortening in normal stromal cells was associated with an increased prostate cancer risk[161]. The findings of these studies need to be validated, but telomere length assessment in the stroma might be particularly useful in men with a negative biopsy but continued suspicion for prostate cancer (for example, owing to persistently elevated PSA levels)[161].

Once prostate cancer has been definitively diagnosed, the currently available prognostic tools cannot reliably predict whether a man with organ-confined disease will or will not eventually develop lethal metastatic disease, particularly in patients with intermediate pathological grade. As a result, many men undergoing treatment are thought to have cancers that would not substantially progress within their lifetimes and could, therefore, forego treatment. The potential harm from the overtreatment of prostate cancer is considerable[162] and new ways to identify the subset of men who will most probably benefit from aggressive treatment need to be identified. Telomere length measurements in biopsy samples could possibly augment current approaches to better inform clinicians and patients whether active surveillance or definitive treatment is more appropriate. In a prospective study using quantitative telomere-specific FISH to assess telomere length, the combination of more variable telomere length in prostate cancer cells and shorter telomere length in cancer-associated stromal cells correlated with progression to metastasis and disease-specific death, independently of existing clinicopathological indicators[105]. These findings indicate a translational potential of tissue-based telomere measurements for prognostication that might inform risk stratification for personalized therapeutic and surveillance strategies.

Telomerase-targeted therapies

Approximately 90% of cancers activate telomerase for telomere maintenance and to achieve unlimited replicative capacity[35,163]. Telomerase is an attractive target for anticancer therapy for two reasons. Firstly, unless cells acquire the ability to maintain telomeres in a telomerase-independent fashion, the lack of telomerase will impair the cancer cell’s ability for unlimited replicative capacity. Secondly, telomerase activation distinguishes normal somatic cells from cancer cells, and normal somatic cells, which lack telomerase, will not be affected by telomerase-targeted therapies.

However, targeting telomerase is not without caveats. Germ cells and some stem or progenitor cells in highly proliferative tissues rely on telomerase to maintain their telomeres; thus, their telomere lengths would be potentially affected by telomerase inhibition causing adverse effects[164]. However, such possible off-target effects were predicted to be minimal, partly owing to the large differential in telomere lengths in cancer versus normal cells. Preclinical studies showed favourable tolerance to telomerase-active agents. As a result, telomerase-directed agents, such as imetel-stat and GV1001, have advanced to clinical trials.

Disappointingly, these treatments have shown no survival benefit, but investigation of their clinical potential has not been entirely abandoned[165] (TABLE 2).

Imetelstat is a lipid-conjugated 13-mer oligonucleotide that functions as a small molecular inhibitor by binding to the RNA template TERC to disrupt telomerase activity. Several, both completed and ongoing, clinical trials have evaluated imetelstat[166]. In patients with non-small-cell lung cancer, imetelstat did not improve progression-free survival; however, tumours with the shortest telomeres responded to treatment better than tumours with longer telomeres, providing support for target-specific efficacy[167]. One notable observation made in a failed phase II trial of imetelstat was a decrease in platelet levels in patients with breast and lung cancer following treatment with this agent[165]. In a small study, imetelstat was found to be particularly efficacious in patients with thrombocythaemia, a hyperproliferative blood disorder characterized by overproduction of platelets by megakaryocytes[168]. Notably, the mechanism of action of imetelstat in patients with thrombocythaemia is unclear, as telomere length was not associated with clinical response[168].

The failure to establish telomerase-targeted treatments for cancer therapy might partly be explained by the fact that telomerase inhibition does not immediately kill cancer cells and is only efficacious when telomeres become critically short. Consequently, the benefits of telomerase inhibition depend on treatment duration, proliferation rate, and initial telomere lengths[169]. Thus, telomerase inhibition might be more effective in highly proliferative cancers or cancers with exceptionally short telomeres. Although prostate cancer is typically a relatively slow growing cancer, the telomeres are mostly substantially shortened. Thus, telomerase inhibition might have clinical utility. Studies in prostate cancer cell lines show that imetelstat causes considerable telomere shortening in the key subset of tumour-initiating cells[170].

Oncolytic virus strategies targeting telomerase- expressing cells are also currently being investigated in clinical trials. Telomelysin is a replication-selective adenovirus in which expression of the viral E1 genes, which are essential for viral replication, are under the control of the *TERT* promoter. The oncolytic virus replicates selectively in cancer cells that express telomerase and not in normal cells lacking telomerase, resulting in selective killing of cancer cells[171]. Completed phase I trials of telomelysin have indicated no severe adverse effects following administration; however, tumour responses in patients have been limited to date[172].

As telomerase-targeted therapies might not be effective as single agents, clinical trials are underway that investigate combination approaches. One study investigating the combination of the telomerase peptide vaccine GV1001 with gemcitabine and capecitabine did not show enhanced efficacy in patients with pancreatic cancer[173]. GV1001 is a 16-mer peptide containing a TERT amino acid sequence. In this study, the peptide was combined with granulocyte–macrophage colony- stimulating factor as an adjuvant[173]. Combination of GV1001 with gemcitabine and capecitabine treatment, which is the standard of care for patients with pancreatic cancer, was thought to potentially enhance the immune response generated by GV1001 vaccination.

The most synergistic combination therapy and optimal delivery method for telomerase inhibition might still have to be identified[165]. In prostate cancer, a potentially efficacious combination therapy inhibits both telomerase activity and AR activity. Wild-type AR in conjunction with p53 represses *TERT* expression in the prostate[133]. However, in prostate cancer, AR signalling acts in an opposite manner and upregulates the expression of TERT[133,135]. Conceivably, this reversal of AR-mediated TERT repression is a consequence of abrogated p53 and AR function. Metastatic castration-resistant prostate cancer is considerably enriched in *TP53* and *AR* alterations compared with organ-confined tumours[174], and increased AR function in the advanced disease setting potentially upregulates telomerase activity. Traditionally, telomerase-targeted treatments have largely focused on the telomere maintenance functions of telomerase; however, compelling evidence suggests additional contributions of TERT in cancer-relevant phenotypes independent of telomere maintenance[175], including the promotion of cell proliferation[176–179], drug resistance[180], and epithelial–mesenchymal transition[181]. In the clinic, prostate cancer responds to antiandrogen treatments, albeit temporarily[182]. Targeting two essential pathways, AR signalling and telomerase, in prostate cancer simultaneously might be a promising strategy to kill tumour cells early and prevent the progression to metastatic disease. The potential synergy of combining AR-targeted and telomerase-targeted therapies warrants preclinical studies.

Therapies directed at shortened telomeres

Therapeutic approaches directed at telomeres have not advanced as far as those targeting telomerase, but warrant further investigation (TABLE 2). A limitation of telomerase-targeted therapies is the potential for cancers to develop resistance via telomerase-independent mechanisms, such as ALT, to maintain telomeres. Only ~10% of all cancers show *de novo* ALT mechanisms[35,163] and no primary prostate cancers exhibit ALT. However, under selective pressure through, for example, telomerase inhibition, telomerase-positive cancers can adopt a telomerase-independent programme of telomere maintenance[183].

When chromosomal ends are not properly protected, numerous proteins associated with the DDR are recruited to the site of telomere dysfunction[20]. In the DDR pathway, sites of DNA lesions, thought to be double-strand breaks, accumulate proteins and modifications to signal for repair. Histone H2AX is phosphorylated at Ser139 (γH2AX) by the kinases ATM, ATR or DNA-PK at the site of the DNA lesion. DNA damage signalling markers such as 53BP1, MDC1, and phosphorylated ATM aggregate at sites of γH2AX to form canonical DNA damage foci[184]. Cancers that employ ALT mechanisms to maintain their telomeres are characterized by a higher level of telomeric DDR compared with telomerase-positive cells[185,186]. One study showed that a small molecule inhibitor of ATR (VE-821) was effective against ALT-positive osteosarcoma and glioma cells[187]. These findings are encouraging for therapy of ALT-positive cancers, but focusing therapeutic efforts on telomeres might require a more comprehensive approach, as targeting telomeres might be efficacious in cancer regardless of telomerase dependency. To develop treatments with maximum therapeutic index, identifying exploitable differences between telomeres in tumours and those in normal cells will be a crucial objective.

Studies in a derivative of the human IMR-90 fibroblast cell line that expresses HPV16 E6 and E7 proteins, which inhibit tumour suppressors p53 and retinoblastoma protein, demonstrated that telomere fusion between chromosomes causes mitotic arrest[188]. Telomere deprotection that arose as a result of prolonged mitotic arrest acted as a molecular signal to trigger cell death. Thus, telomere deprotection might sensitize cancer cells to antimitotic drugs[189]. Intriguingly, a sub-population of prostate tumour cells with elevated telomerase activity and *TERT* expression were sensitive to strategies inducing apoptosis through telomere deprotection[190]. Expression of mutated TERC in these cells reprogrammed telomerase to extend telomeres with an incorrect sequence, effectively inducing telomere deprotection. Therapeutic strategies that promote telomere deprotection, such as G-quadruplex stabilizers[191,192], might have clinical utility in prostate cancer, particularly in the setting of metastatic castration-resistant disease, in which p53 inactivation is common[174].

Disruption of AR function in AR-positive prostate cancer cells results in telomere dysfunction, and activates the DDR proteins ATM and checkpoint kinase 2 (REF. 193). Mutational studies of the shelterin protein TIN2 showed that deletions in the C-terminal or N-terminal regions of TIN2 triggered cell death in the AR-negative and p53-deficient PPC-1 prostate cancer cell line but not in the AR-positive and p53-positive LNCaP prostate cancer cell line, suggesting that the telomere complex might be different between AR-negative and AR-positive prostate cancer[194]. Consistent with this notion, treating AR-positive LNCaP cells with the AR antagonists bicalutamide or enzalutamide resulted in γH2AX enrichment and recruitment of 53BP1 at telomeres. However, telomere dysfunction was not observed following bicalutamide treatment in the AR-negative PC-3 cell line[188]. Furthermore, AR inactivation by knockdown, androgen deprivation, or treatment with bicalutamide in LNCaP cells induced telomere breaks and sister chromatid telomere fusion[195]. Blocking the repair of these telomere DNA lesions with an ATM inhibitor enhanced cell killing by bicalutamide in both LNCaP (androgen-responsive) and CWR 22Rv1 cells (androgen-insensitive). In this setting, ATM inhibition blocked cell cycle checkpoint arrest, preventing the repair of damaged telomeres caused by AR inhibition, and as a result promoted cell death[193]. These studies suggest that combination treatments including an ATM inhibitor that potentiates the effects of existing androgen deprivation therapies are effective via telomere-directed mechanisms and might have clinical utility in both androgen-sensitive and androgen-insensitive prostate cancer.

Conclusions

Telomeres and telomerase seem to be integral to the initiation and progression of prostate cancer and, therefore, might also be relevant in the diagnosis, prognosis, and treatment of the disease (FIG. 3). At the earliest stages of prostate tumorigenesis, telomere shortening can be observed in the precursor lesion HGPIN. The exact causes of telomere shortening in the prostate are not yet well defined, but evidence suggests an inflammatory contribution that increases local ROS production, which accelerates telomere shortening in addition to the losses caused by cell proliferation.

Critically shortened telomeres compromise genomic integrity and can drive somatic copy number alterations, aneuploidy, and DNA rearrangements that are typically observed in the

genomic landscape of prostate cancer. The ensuing genomic instability contributes to the somatic molecular alterations to sustain the unlimited replicative capacity in cancer. Essential to this process is the activation of telomerase or the adoption of telomerase-independent mechanisms to maintain telomeres. For the majority of prostate cancers, MYC is believed to be the main driver for telomerase activation.

The combination of short telomeres and telomerase activation in prostate cancer make telomerase an attractive therapeutic target. However, in clinical trials, telomerase-targeted therapies for other solid cancers have mostly proven to be ineffective as single agents. Clinical studies have not fully investigated the use of these treatments in prostate cancer, but the outcomes of single-agent treatment will probably be similarly disappointing. The utility of telomerase-targeted therapies in prostate cancer possibly exists in combination with other established treatment regimens, such as AR inhibitors or radiotherapy. In addition, a subset of lethal metastatic prostate cancer has been shown to employ the telomerase-independent telomere maintenance programme ALT for telomere maintenance. The potential for prostate cancer to employ ALT is particularly salient, as this mechanism is a way for advanced prostate cancer to circumvent telomerase-directed therapies and continue to maintain and extend telomeres. Thus, inhibiting telomerase activity might not be sufficient to treat advanced prostate cancer, as ALT mechanisms might become engaged to maintain telomeres and support continued cell division. New findings that connect telomere dysfunction and AR inhibition in prostate cancer cells are provocative, and re-emphasize the importance of the telomeres as a potential therapeutic target in prostate cancer. Telomere-directed therapies in combination with AR inhibition, perhaps also including targeting of the DDR, might have the potential to be effective in both telomerase-expressing and ALT-positive prostate cancers.

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Glossary

T-Loop
A structure stabilized by shelterin proteins at the end of telomeres, where the telomere double-stranded DNA loops onto itself to form a partial overlap between the 3′ G-rich telomere overhang and the complementary C-rich telomere strand upstream of the overhang.

End replication problem
During DNA replication, synthesis on the lagging DNA strand of linear templates is incomplete, resulting in the loss of ~50 terminal nucleotides in each round of cellular division.

Replicative senescence
In normal cells, cessation of cell division owing to substantial telomere shortening following ~50 cell divisions (Hayflick limit).

BPH
Noncancerous enlargement of the prostate owing to hyperproliferation of epithelial and/or stromal cells in the prostate.

Prostatic intraepithelial neoplasia (PIN)
A noncancerous lesion in the prostate with abnormal acinar architecture, observed as overcrowding of luminal cells with enlarged nuclei.

High-grade PIN (HGPIN)
Considered a precursor lesion of prostate cancer, featuring cancer-like morphological abnormalities (for example, nuclear pleomorphism and prominent nucleoli), but no evidence of invasion.

Reactive oxygen species (ROS)
Highly reactive, oxygen-containing free radicals that can damage cellular RNA, DNA, and proteins.

8-Oxoguanine
The best-characterized and highly abundant DNA lesion arising from the oxidation of guanine through reactive oxygen species.

Base excision repair (BER)
The DNA repair pathway that employs specialized DNA glycosylases, N-glycosylase/DNA lyase and adenine DNA glycosylase, to repair 8-oxoguanine.

G-Quadruplexes
Nucleic acid secondary structures arising from Hoogsteen base pairing (an alternative form of base pairing) interactions of guanine residues.

Fragile sites
Unstable regions in the genome that are prone to break under replication stress.

Prostatic inflammatory atrophy
Prostatic lesions characterized by increased proliferation and atrophic morphology of prostatic luminal epithelial cells, associated with local inflammatory cells.

Chromothripsis
Multiple translocation events occurring in a single catastrophic event leading to imperfect rearrangement and repair of one or a few shattered chromosomes.

Overdiagnosis and overtreatment
Diagnosing patients with a disease that will not give rise to symptoms or cause death, often leading to treatment that might have no benefit and might even be harmful to the patient.

Fluorescence *in situ* hybridization (FISH)
A technique using fluorophore-conjugated oligonucleotide probes that bind to specific DNA sequences via complementary Watson–Crick base pairing, enabling detection of sequences of interest in intact cells or chromosomes by fluorescence microscopy.

Prostate Cancer Prevention Trial

A study conducted from 1994–2003 to investigate if the 5α-reductase inhibitor finasteride reduces prostate cancer development in men ≥55 years of age.

Peptide vaccine

A peptide conjugated with a vaccine adjuvant to stimulate an immune response against a target antigen that shares the same amino acid sequence of the peptide.

Epithelial–mesenchymal transition

The biological process in which epithelial cells acquire characteristics more consistent with mesenchymal cells, including loss of cell polarity and adhesion, and enhanced migration and invasiveness.

Telomere deprotection

Telomeres partially or completely unprotected by shelterin proteins, resulting in the activation of DDR.

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Key points

- Telomerase activation or the cancer-specific, telomerase-independent alternative lengthening of telomeres (ALT) mechanism are two telomere maintenance mechanisms in human cells. Most prostate cancers activate telomerase and a subset of lethal metastases use ALT
- Substantial telomere shortening is common in prostate cancers and in the precursor lesion prostatic intraepithelial neoplasia (PIN). Moderate telomere shortening has also been observed in cancer-associated stroma
- The mechanisms for telomere shortening in prostate cancer and PIN are not fully understood; in addition to replication-associated telomere loss, inflammation and reactive oxygen species might be contributors
- Telomere length assessment might be useful in prostate cancer diagnosis and in current prognostic tools to more reliably predict whether organ-confined prostate cancer will progress to lethal metastatic disease
- Telomerase-targeted single-agent treatments for solid cancers have, to date, been ineffective in clinical trials; these therapies have yet to be tested in prostate cancer and might potentially be useful in combination with established androgen receptor (AR)-targeted treatments
- Disruption of AR function in AR-positive prostate cancer cells activates the DNA damage response (DDR) at telomeres; thus, DDR inhibitors might potentiate the effects of androgen deprivation therapy

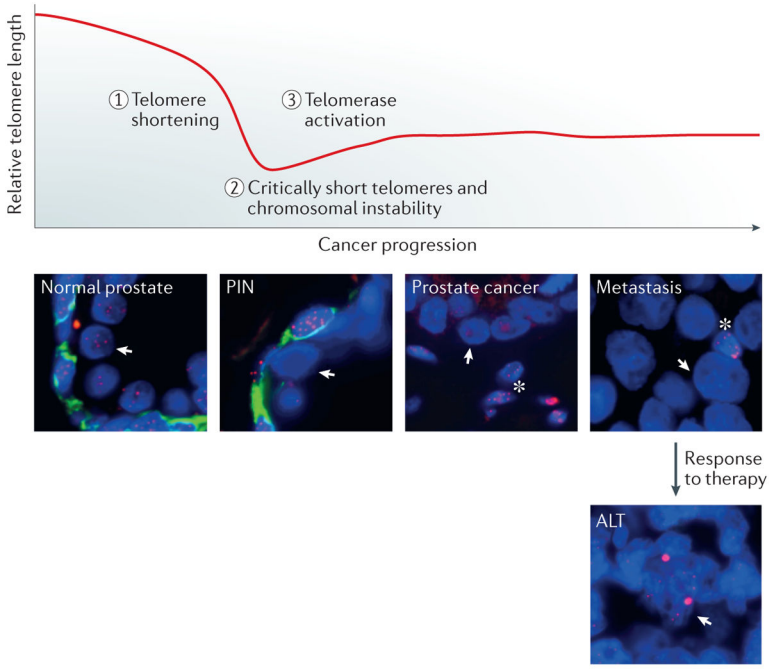

Figure 1. Telomere shortening during prostate tumorigenesis and cancer progression

Telomeres gradually shorten in each round of cell division owing to incomplete replication of the lagging DNA strand during DNA synthesis. To sustain unlimited replicative capacity, prostate cancer cells activate telomerase; however, they maintain short telomeres during prostate tumorigenesis and cancer progression, which can be seen in biopsy samples following fluorescence *in situ* hybridization staining (telomeres: red, Cy3-labelled anti-telomeric probe; basal cells: green, anti-cytokeratin antibody 34bE12; DNA: blue, DAPI). In the normal prostate, telomere lengths are relatively similar between luminal (arrows) and basal cells. However, in prostatic intraepithelial neoplasia (PIN), telomere staining in the luminal cell is substantially less than in the basal cells, indicative of telomere shortening in the luminal compartment. In prostate cancer, which lacks basal cells, the telomere staining is substantially less than in neighbouring stromal cells (asterisks). Likewise, in cancer metastases, telomere staining is less than in infiltrating lymphocytes (asterisks, reduced nucleus size). In a subset of metastatic prostate cancer cells, alternative lengthening of telomeres (ALT) mechanisms are activated, possibly as a response to treatment or environmental factors. Bright telomeric DNA foci are characteristic of ALT.

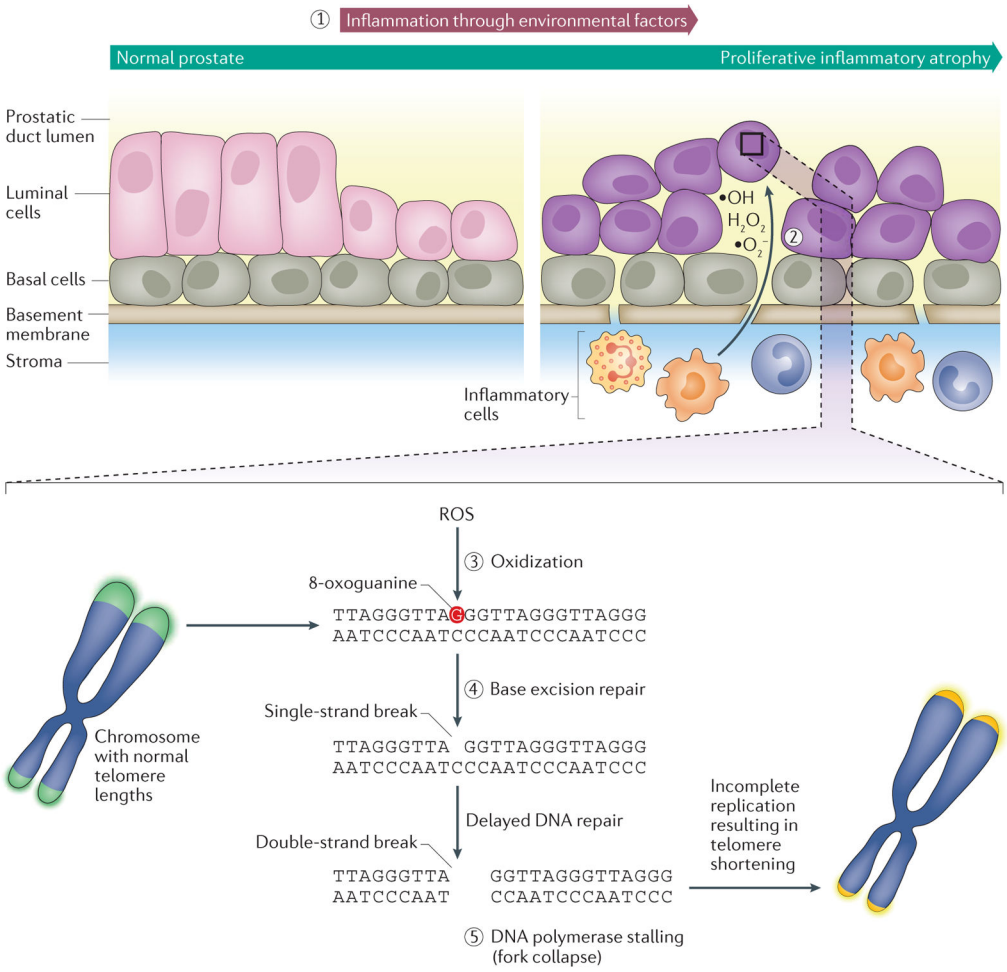

Figure 2. Reactive oxygen species as a cause of telomere shortening in prostate tumorigenesis

Prostatic inflammation is common, can result in the formation of prostatic inflammatory atrophy (PIA), and has been associated with prostate cancer (1). PIA lesions are characterized by an increased proliferation of epithelial cells and contain activated inflammatory cells (predominately lymphocytes and macrophages), which produce reactive oxygen species (ROS), such as $·O_2^-$, H_2O_2, and ·OH (2). ROS can cause oxidization of guanine to form 8-oxoguanine (3), and incomplete base excision repair of 8-oxoguanine (4) can trigger DNA polymerase replication stalling and replication fork collapse at telomeres (5). Because DNA damage response is repressed at chromosomal ends, resulting in incomplete telomere replication, telomeres are vulnerable to severe and unmitigated telomere erosion.

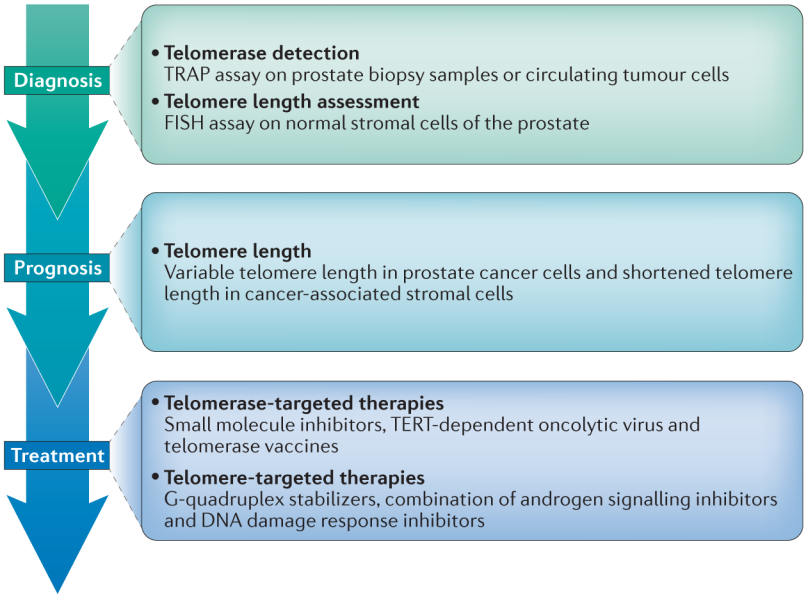

Figure 3. Potential applications directed at telomeres and telomerase in prostate cancer management

At the diagnostic stage, detection of telomerase expression and measurement of telomere length might be useful, as telomerase activation and shortened telomeres are strongly associated with prostate cancer. At the prognostic level, determining telomere lengths might enable distinguishing men who will develop lethal metastatic disease from men whose disease is unlikely to advance beyond an organ-confined stage. At the therapeutic level, strategies targeting telomerase or telomeres might have clinical utility, particularly in combination with traditional prostate cancer therapies that target the androgen signalling pathway.

Table 1

Cancer-associated activation of telomerase

Molecular mechanism	Prostate cancer relevance	Refs
TERT amplification	Amplification of *TERT* has not been observed in prostate cancer, but has been reported in embryonal tumours of the central nervous system, cervical carcinoma, lung adenocarcinoma, and hepatocellular carcinoma.	196–199
TERT translocation	Genomic rearrangements of *TERT* have not been reported in prostate cancer, but have been tightly linked to high-risk neuroblastoma.	200,201
TERT promoter hypermethylation	The methylation status of the *TERT* promoter in prostate cancer has yet to be fully investigated, but promoter methylation has been reported to occur in multiple cancer cell lines and in some cancers.	137,202
TERT promoter activating mutations	*TERT* promoter mutations have not been observed in prostate cancer, but have been frequently observed in cancers of the central nervous system, bladder, thyroid, and skin.	138,139
MYC activation	MYC positively regulates *TERT* expression and MYC activation coincides with the earliest detectable appearance of abnormally short telomeres during prostate tumorigenesis. >80% of prostate cancers have nuclear overexpression of MYC. Thus, MYC activation is likely to be a mechanism of TERT activation in a high proportion of prostate cancers.	141,203

Table 2

Therapies directed at telomerase or telomeres

Mechanism of action	Example	Prostate cancer relevance	Refs
Telomerase inhibition with small molecules	Imetelstat is a lipid-conjugated 13-mer oligonucleotide that functions as a small molecular telomerase inhibitor by binding to the RNA template, TERC, and disrupts telomerase activity.	Clinical trials in breast and lung cancers have not been successful. No clinical trials of telomerase small molecule inhibitors in prostate cancer exist, but preclinical studies of prostate cancer cell lines show that imetelstat causes telomere shortening in tumour-initiating cells.	167,169
Telomerase vaccine	GV1001 is a 16-mer peptide vaccine containing a TERT amino acid sequence used to elicit immune responses against cells with telomerase activity.	No studies have investigated the use of GV1001 in prostate cancer to date. In pancreatic cancer, clinical trials of the vaccine have shown the agent to be immunogenic and well tolerated in patients; however, GV1001 was not efficacious as a single agent or in combination therapies.	173,204
TERT-regulated oncolytic virus	Telomelysin is a replication-selective adenovirus engineered to express the essential viral E1 replication genes under the control of the *TERT* promoter.	Telomelysin was effective in a LNCaP tumour model in nude mice. Completed phase I trials of telomelysin in various solid tumours (not including prostate cancer) have indicated no severe adverse effects following administration, but patient tumour response was limited.	172,205
Telomerase inhibition through AR down regulation	AR inhibitor bicalutamide and methaneseleninic acid downregulate AR protein level	AR inhibition using methaneseleninic acid in combination with bicalutamide decreased *TERT* expression and increased apoptosis in prostate cancer cells.	136
Telomere deprotection	G-Quadruplex stabilizers and telomestatin	No G-quadruplex stabilizers that specifically target telomeres have advanced to clinical trials. Quarfloxin, which has advanced to clinical trials, does not interact with telomeres, but with ribosomal DNA G-quadruplexes in the nucleolus. Telomestatin disrupts the interaction of shelterin protein TRF2 with telomeres in glioma stem cells, but the effects in prostate cancer have not been investigated. Prostate cancer mouse models responded to treatment with TMPyP4 and RHPS4.	206–210
Telomere dysfunction through AR inhibition	AR inhibitor (for example, bicalutamide or enzalutamide) and ATM inhibitor (KU-60019)	In prostate cancer cell culture studies, AR inhibition resulted in telomere dysfunction. ATM inhibition blocked cell cycle checkpoint arrest and prevented the repair of damaged telomeres caused by AR inhibition, promoting cell death.	193

AR, androgen receptor; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase.