# Insights into telomerase activity in bladder cancer pathology

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**Submitted:** 20 May 2025; **Accepted:** 4 November 2025
**Online publication:** 28 January 2026

Arch Med Sci
DOI: https://doi.org/ 10.5114/aoms/213923


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## Abstract

Bladder cancer (BC) is among the top ten most commonly diagnosed cancers worldwide, with rising incidence and around 213,000 deaths annually. It exhibits a marked male predominance, largely attributable to risk factors such as tobacco use, occupational carcinogen exposure, and genetic predisposition. Bladder cancer is classified into non-muscle-invasive (NMIBC) and muscle-invasive (MIBC) forms, with the latter linked to worse prognosis. Despite current treatment modalities, including surgical resection with adjuvant chemotherapy or immunotherapy, disease management remains challenging due to high recurrence rates and limited therapeutic efficacy in advanced stages. Recent studies highlight telomerase activity, especially TERT promoter mutations present in about 70% of BC cases, as a key contributor to disease progression. These findings position telomerase as a promising biomarker for early detection, disease monitoring, and targeted therapy. This review summarizes recent advances in understanding telomerase regulation in BC and its potential clinical applications.

**Key words:** bladder cancer, telomerase activity, prognostic biomarkers, diagnostic biomarkers.

## Introduction

Bladder cancer (BC) is one of the most prevalent malignancies in adults, with both incidence and mortality rates increasing. As a major global health problem, it ranks among the top ten most commonly diagnosed tumors, causing an estimated 213,000 deaths per year [1]. Due to risk factor exposure differences, including smoking, occupational chemicals, hormonal, and genetic variations, BC is significantly more common in men than women. It ranks as the sixth most prevalent tumor among men and the sixteenth among women [2, 3]. A key contributor to this global burden is tobacco smoking, which accounts for nearly half of cases and increases the risk approximately threefold, particularly among individuals over the age of 55 [4, 5]. Additionally, geographic variations indicate the influence of environmental and genetic factors, revealing that Europe has higher incidence rates, while Asia has lower rates. Furthermore, occupational exposure to carcinogenic chemicals, particularly in industries such as chemical manufacturing, rubber, and dye production, has been identified as a significant risk factor [6, 7].

Bladder cancer is divided into two main types that develop from the epithelium of the urinary tract: non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Compared to NMIBC, MIBC is characterized by a higher degree of advancement and a worse prognosis, because of higher metastatic risk [8]. Survival decreases with disease progression, ranging from 96% after five years for in situ cancer to only 6% for cases with distant metastases [9]. Therefore, early diagnosis of the disease significantly impacts prognosis: patients diagnosed at an earlier stage have a lower incidence of muscle layer involvement and a better prognosis. The aggressiveness and prognosis of BC depend primarily on the degree of invasion of the bladder wall and the stage of the tumor at the cellular level. According to traditional American Joint Committee on Cancer (AJCC) tumor-node-metastases (TNM) staging, BC are categorized into MIBC and NMIBC according to the level of invasion as well as into low-grade and high-grade based on cellular properties [10, 11]. In addition, the histopathological categorization of BC is one of the most reliable factors in determining tumor biology, which can improve treatment planning. Table I presents various stages of BC. Although the prognosis of BC may vary, the degree of tumor invasion of the bladder wall is a key factor in risk assessment and selection of treatment strategy.

Currently, the conventional treatment for BC involves surgical resection combined with adjuvant chemotherapy and immunotherapy [8]. Despite significant advancements in diagnosis and treatment, BC remains a major clinical challenge. This is due to its high recurrence rate after surgery and the limited efficacy of therapies available for advanced stages of the disease. One of the key molecular mechanisms associated with the progression and potential recurrence of BC is telomerase activity [12, 13]. Among these mechanisms, the leading role is played by mutations in the promoter of the gene encoding telomerase reverse transcriptase (*TERT*), which occur with a frequency of 70–80% among patients with BC. The discovery of common mutations creates opportunities for new therapeutic approaches and facilitates early detection and monitoring of the disease after treatment through urine and blood analyses. Despite numerous studies, the mechanisms regulating telomerase activity in BC remain incompletely understood. In particular, the role of telomerase gene regulation and the interactions between telomerase and other cancer cell signaling pathways still require further investigation. Understanding these mechanisms may provide new diagnostic and therapeutic options that could revolutionize management of BC.

The aim of this review is to present the latest research on the role of telomerase and TERT promoter mutations in BC. The review summarizes the mechanisms of telomerase regulation in the context of BC progression and analyzes its potential as a diagnostic marker and therapeutic target.

A rigorous methodology based on narrative review guidelines was used to create a comprehensive review critically examining the role of telomerase in BC. The literature search included databases such as PubMed, Scopus, and Google Scholar. Combinations of keywords were used, including "bladder cancer," "telomerase," "TERT promoter," "biomarker," "cancer progression," "targeted therapy," and "regulatory mechanisms." The search was limited to articles published in English, with priority given to publications from peer-reviewed journals. Original studies and systematic reviews were included in the analysis, while conference abstracts and letters to the editor were excluded. After screening, the full-text articles were assessed for relevance and quality. The review is narrative, based on synthesis and critical interpretation of the selected works.

## Biology of telomeres

Telomeres are specialized complexes that protect the ends of chromosomes against degradation and fusion, which translates into maintaining genome stability [14]. By participating in the spatial organization of the cell nucleus and transcriptional regulation at subtelomeric loci, they prevent chromosome aberrations and en-

**Table I.** Main stages of non-muscle and muscle invasive bladder cancer histology according to TNM classification system [10]

| Non-muscle invasive bladder cancer | | Muscle invasive bladder cancer | |
|---|---|---|---|
| Ta | Non-invasive papillary carcinoma | T2 | Tumor invasion of muscle<br>T2a – superficial muscle invasion<br>T2b – deep muscle invasion |
| Tis | Urothelial carcinoma in situ | T3 | Tumor invasion of perivesical tissue<br>T3a – microscopically<br>T3b – macroscopically |
| T1 | Tumor invasion of subepithelial connective tissue | T4 | Tumor invasion of perivesical organs<br>T4a – prostate, uterus, vagina invasion<br>T4b – pelvic or abdominal wall invasion |

able the proper course of recombination [15]. The length of telomeres varies across cells. They shorten with each cell division because telomeres cannot be fully replicated during DNA replication. This is a natural, age-related process, and excessively short telomeres trigger processes such as cellular senescence, apoptosis, and cancerogenesis. In human cells, telomeres are composed of non-coding nitrogenous bases repeats with the sequence 5′-TTAGGG-3′. Their structure includes long double-stranded DNA fragments (10–15 kb long) rich in cytosine, terminating in the final guanine-rich 3′ single-stranded DNA (150–200 nucleotides long) [16]. This overhanging 3′-OH strand is crucial for the formation of specific structures such as the T-loop and the D-loop (Figure 1). The T-loop structure is one of the most prominent features of telomeres, protecting the ends of chromosomes from being recognized as DNA damage. It is formed when the overhanging G strand of 3′-OH inserts into the double-stranded region of telomeric DNA, creating a lasso-like structure. This specific DNA organization is crucial for the protective function of telomeres because it masks the end of the chromosome, preventing the activation of repair mechanisms that could lead to chromosome fusion or other genetic catastrophes. Inside the t-loop is the D-loop, a structure formed by the G strand entering a double-stranded section of telomeric DNA. The D-loop stabilizes the T-loop and additionally protects telomeres against damage [17, 18]. Telomeric DNA in the large T-loop is bound by the six-member protein complex called shelterin, forming a telomeric capping structure. The complex has three main subunits: telomeric repeat factor 1 and 2 (TRF1, TRF2), which recognize and bind double-stranded TTAGGG repeats, and telomere protection protein 1 (POT1), responsible for binding single-stranded TTAGGG overhangs [19]. These three proteins are integrated through TRF1-interacting protein 2 (TIN2), TPP1 protein, and repressor-activator protein 1 (RAP1), which function as intermediary proteins between binding proteins telomeric DNA.

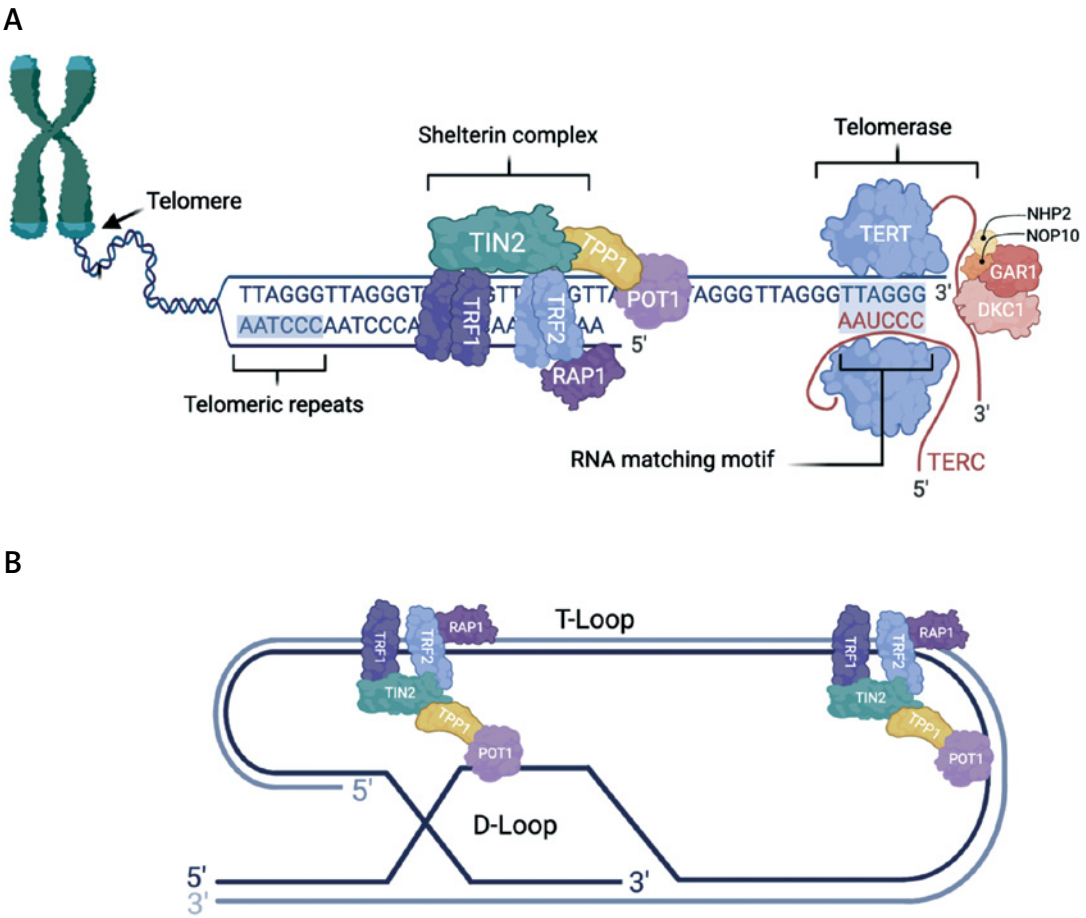


**Figure 1.** Telomere structure maintenance. **A** – Telomere-binding protein complex. Human telomeres are composed of 5–12/15 kb TTAGGG repeats. Telomerase is a ribonucleoprotein complex that maintains their length during cell division. The shelterin complex regulates telomere structure and consists of six proteins: TRF1, TRF2, POT1, RAP1, TIN2, and TPP1. TRF1 and TRF2 bind to double-stranded DNA, POT1 and TPP1 bind to a 3′ single-stranded end, RAP1 binds to TRF2, and TIN2 stabilizes the interactions in the complex. **B** – Telomeric T-loop and D-loop structure. Telomeres form a T-loop (telomeric), and the guanine-rich 3′ end inserts into the duplex repeats to form a D-loop (displacement). These structures are stabilized by the shelterin complex, providing protection to the telomeres

## Telomerase structure and function

Telomerase is a ribonucleoprotein enzyme that plays a key role in maintaining chromosome integrity and thus genome stability by extending telomeres, the terminal segments of chromosomes, that shorten during each cell division. In most somatic cells, telomerase activity is tightly regulated, and its absence leads to telomere shortening, causing cellular senescence [20]. The main components of telomerase holoenzyme include human telomerase reverse transcriptase (h*TERT*) and human telomerase RNA component (h*TERC*, *hTR*) [21].

The catalytic subunit h*TERT* is responsible for the enzymatic activity that adds repetitive nucleotide sequences to telomeres, thereby counteracting the progressive shortening of telomeres that occurs during DNA replication [22]. It is organized into distinct domains such as the RNA-binding domain (TRBD), responsible for facilitating the interaction with the telomerase RNA component; the reverse transcriptase (RT) domain, which performs the polymerization of DNA nucleotides; and the C-terminal extension (CTE), which contributes to the structural stability and enzymatic function of telomerase. The active site of h*TERT* contains highly conserved aspartate residues, which are essential for its enzymatic function. Any mutation in these residues can disrupt telomerase function and impair telomere replication [8]. The h*TERC* subunit is a long non-coding RNA that binds to the 3′ ends of chromosomes to form a template for telomeric DNA synthesis, ensuring the accuracy and efficiency of the elongation process [23]. This subunit is vital for the structural integrity and functional capabilities of the telomerase enzyme complex. In addition to serving as a template of telomeric DNA synthesis, the *TERC* subunit also functions as a structural scaffold, facilitating the binding of 32 different accessory proteins such as dyskerin (*DKC1*), *GAR1*, *NHP2*, and *NOP10*, which are crucial for the proper assembly and stability of the telomerase holoenzyme [24]. The *TERC* subunit exhibits a range of structural conformations, including open and closed states, which are thought to influence telomerase catalytic activity and regulation [25]. Furthermore, the correct folding of specific domains, such as *CRC4/5* (within *TERC*), is essential for the binding of accessory proteins and enzyme activity. Chaperones such as *TCAB1* bind to the telomerase RNA component and play a critical role in the folding process. The intricate relationship between telomerase structure and function is key to understanding its role in various human diseases, including bone marrow failure, pulmonary fibrosis and cancer diseases, where its aberrant activation enables uncontrolled cell proliferation and tumor progression [26]. Telomerase mutations or dysregulation promote oncogenesis by preventing telomere shortening and allowing cancer cells to proliferate without restriction. Telomerase has therefore become a target for anticancer therapies, including inhibitors, immunotherapies, and gene editing.

## Mechanism of telomerase activation in normal and cancer cells

Telomerase activity is finely regulated in humans through a complex. The enzyme is active during embryogenesis; however, after birth its function is inhibited in most somatic cells. Its activity is maintained only in specific subsets of cells, such as stem cells, germ cells, progenitor cells, hematopoietic cells, and activated lymphocytes. However, *TERT* is not expressed in most healthy cells, and thus it can serve as a biomarker for cancer. In addition, normal cells have a certain number of divisions they can undergo during their lifetime, known as the Hayflick limit. This limit varies depending on the degree of cell differentiation in each population (except for stem cells). Programmed cell death through apoptosis, which occurs after reaching the limit of division, is a natural protection against cancer [27]. Achieving immortality is therefore a key condition for the development of cancer and a necessary element of its malignancy. Resistance to programmed cell death mechanisms is one of the hallmarks of cancer cells, acquired mainly by telomerase activation or alternative lengthening of telomeres (ALT) [28].

The most common mechanism leading to unlimited cell division and thus the immortality of neoplastic cells is excessive activation of the telomerase enzyme. Its activation together with maintenance of telomere length is found in 90% of diagnosed human cancers [13, 29]. Aberrant telomerase activation occurs in most cancers, allowing cells to avoid senescence. Telomerase reverse transcriptase, a key component, is regulated by various signaling pathways, such as MYC, Wnt/β-catenin, PI3K/AKT/mTOR and NF-κB pathways [30]. These mechanisms include post-translational modifications (PTMs), interaction with telomeric repeat-containing RNA (*TERRA*), and regulation by telomerase interacting protein 1 (PINX1). Changes in the *TERT* gene promoter and its phosphorylation lead to its increased expression at both the transcriptional and protein levels. High telomerase activity has been found in many types of cancer, including BC. In healthy tissues, *TERT* expression is tightly regulated and active mainly in stem and germline cells, where it supports tissue regeneration and repair. In contrast, its aberrant expression in somatic cells is a hallmark of many cancers, facilitating uncontrolled proliferation by allowing cells to bypass normal

senescence pathways [31]. Statistically significant differences in *TERT* expression have been observed when comparing cancer tissues with their healthy counterparts. Rathi *et al.* highlighted its overexpression in liver cancer as a critical contributor to tumor growth and survival [32]. In addition, *TERT* gene promoter mutations have been found in about 70% of patients with BC, suggesting their role in the initiation of the neoplastic process, similarly, Werr *et al.* reported that its activation was associated with a more aggressive course of lung cancer [33, 34].

## Telomerase aberration in bladder cancer

The *TERT* gene is located on chromosome 5p15.33 and consists of 16 exons and 15 introns of 35 KB [29]. Its key promoter is one of the most frequently described mutation sites in studies. The promoter mutations predominantly occur at two hotspots, C250T and C228T (positions 1,295,250 C > T and 12,952,228 C > T) located –124 and –146 bp upstream of the ATG initiation codon, respectively [35]. These mutations are among the most frequently observed in various cancers, including BC, and are associated with the activation of telomerase, contributing to tumorigenesis and poor prognosis. The C228T and C250T mutations create de novo binding sites for erythroblast transformation specific (ETS) transcription factors, such as GA-binding protein (GABP), leading to increased TERT transcription and telomerase activity. This reactivation of telomerase is a critical step in cellular immortalization and cancer progression [36].

Furthermore, studies have shown a correlation between high *TERT* expression and increased tumor invasiveness and metastatic potential, further emphasizing its critical role in cancer pathology. As BC progresses from a noninvasive to an invasive stage, *TERT* gene expression tends to increase, suggesting its role in facilitating tumor growth and invasion. Higher levels of its expression have been observed in muscle-invasive BC compared to non-muscle-invasive forms, highlighting its potential as a biomarker of disease severity. This correlation highlights the importance of monitoring *TERT* expression in clinical practice to better understand and treat BC progression [37, 38].

Studies of human telomerase RNA component expression in tumors and healthy tissues have revealed important insights into its potential role in tumorigenesis and progression [39]. Higher levels of *hTR* expression have been shown to be frequently observed in advanced stages of BC, suggesting a role in tumor progression [29]. Its elevated expression levels are associated with more aggressive forms of cancer, including gastric and BC. This suggests that *hTR* may contribute to the increased proliferative capacity of tumor cells, allowing them to evade normal growth controls and resulting in more aggressive tumor behavior. Furthermore, the association between *hTR* expression and BC stages highlights the involvement of the gene in the molecular mechanisms driving cancer progression.

Mutations in the *hTR* gene are diverse and can significantly affect its function, leading to increased telomerase activity or dysfunction depending on their nature and location. These mutations include point mutations, deletions, and insertions that alter the sequence of the RNA template [40]. The introduction of mutations in the *hTR* sequence can affect its increased expression or prevent its inhibitors from recognizing and then blocking it [41]. On the other hand, mutations that disrupt *hTR* function can lead to telomere shortening and subsequent cellular senescence or apoptosis, acting as a barrier to tumorigenesis in some contexts [13]. However, in cancer cells, the presence of *hTR* mutations often correlates with more aggressive disease phenotypes. These cells can maintain their telomeres despite genetic changes, thus supporting continued proliferation and survival. This dual role of hTR mutations highlights the complexity of their involvement in cancer, emphasizing the potential of the gene as both a therapeutic target and a biomarker of cancer prognosis.

*DKC1*, a component of the telomerase complex, is upregulated in various human cancers, highlighting its potential role in oncogenesis [42]. Furthermore, its high expression has been associated with poor prognosis, partly due to its role in maintaining elevated hTR levels [43]. Elevated *DKC1* expression levels are often associated with poor prognosis and reduced survival of patients, as it may affect the immune infiltration of tumor cells. Studies have shown significantly higher levels of its expression in cancer tissues compared to normal tissues. In addition, it is highly expressed in colorectal cancer tissues compared with adjacent healthy tissues [44, 45]. These results suggest that high *DKC1* expression in cancer tissues may be a common feature of many types of cancer, potentially contributing to the malignant phenotype. *DKC1* undergoes multiple genetic alterations in diverse types of cancer, with missense and application mutations being the most common [46]. In BC, these mutations can disrupt normal cellular processes, contributing to tumorigenesis and cancer progression. Mutations in the *DKC1* gene can significantly affect BC progression by altering the translation of mRNAs that encode key tumor suppressor proteins such as p27 and p53 [47]. These tumor suppressors are critical in regulating the cell cycle and apoptosis, and their impaired function due to *DKC1* mutations can lead to un-

controlled cell growth and cancer development. An increased *DKC1* copy number significantly increases the overall expression of this gene, which directly affects the activity of the telomerase complex. Other studies have shown an altered pattern of *DKC1* methylation in many types of cancer, which translates into a pattern of its expression and increased telomerase activity [48, 49]. Consequently, these genetic changes contribute to tumor progression and poor prognostic outcomes in patients with BC.

## Regulation of telomerase genes in bladder cancer

The regulatory mechanisms of the telomerase gene, particularly human telomerase reverse transcriptase, in BC cells are multifaceted and involve complex layers of transcriptional and epigenetic control. Transcriptional regulation is a key element, as it provides potential therapeutic targets for inhibiting telomerase activity in cancer cells, thereby limiting their proliferative capacity [50]. Epigenetic modifications, such as histone deacetylation and h*TERT* promoter methylation, play a significant role in the regulation of *TERT* expression. Central to this regulation are specific transcription factors that bind to the core promoter region of the *TERT* gene, as well as to other regulatory elements within its promoter [51].

Among the key transcription factors, c-Myc stands out as a significant activator of *TERT* expression, making it a potential therapeutic target in BC. c-Myc forms heterodimers with Max proteins (also known as myc-associated factor X), directly activating the h*TERT* promoter, increasing the transcription of the telomerase gene [52]. Furthermore, the interaction of c-Myc with factor Sp1 is crucial, as it binds to responsive elements within the core promoter, thereby enhancing transcriptional activation of *TERT*. It is worth noting that c-Myc also affects the activity of Ets1 and Ets2 proteins, which can modulate hTERT expression as activators (e.g. c-Myc, Max, Sp1, Ets1, Ets2) or inhibitors (e.g. Mad1, Wt1, p53), depending on the specific DNA sequences with which they interact.

The relationship between telomerase and Myc signaling is multifaceted and complex, involving both direct and indirect mechanisms that modulate *TERT* transcription and activity. One of the key aspects of this relationship is the role in regulating *TERT* expression, where Myc can act as an activator or repressor of *TERT* transcription depending on the cellular context [53]. Heterodimeric Myc/Max complexes bind specifically to E-boxes in the *TERT* promoter, inducing topological changes in DNA that facilitate transcriptional activation. However, this relationship is not universally consistent across cancer types. Studies in breast cancer and hepatocellular carcinoma have not shown a significant correlation between Myc and *TERT* expression, suggesting the presence of additional regulatory mechanisms that may act independently of Myc in these contexts. Conversely, in Myc-driven cancers such as lymphoma, a feedback loop has been observed in which Myc increases *TERT* transcription and in turn stabilizes Myc protein levels, thereby promoting oncogenesis. This interaction highlights the complexity of Myc and *TERT* interactions, reinforcing the need for further studies to uncover the underlying molecular mechanisms and their implications in cancer biology.

The complex relationship between *TP53* and telomerase activity is crucial to understanding cancer development and progression. *TP53*, commonly known as p53, acts as a tumor suppressor protein that plays a key role in maintaining genomic stability. One of the key mechanisms by which p53 exerts its effects is through the regulation of telomerase activity. Studies have shown that loss or inactivation of p53 can lead to the activation of the telomerase enzyme, thereby promoting cellular immortality and tumorigenesis [54]. Furthermore, p53 is involved in the cellular response to telomere dysfunction; its activation can induce cell cycle arrest or apoptosis in response to critically short telomeres, thereby preventing the propagation of genomic instability [55].

Another layer of regulation is provided by epigenetic modifications, such as methylation and demethylation processes, which can affect the accessibility of the promoter for transcription factors. Methylation at CpG islands in the *TERT* promoter region can inhibit transcription factor binding, thereby repressing *TERT* expression [56]. These complex layers of transcriptional and epigenetic regulation underscore the complexity of *TERT* expression control, emphasizing the need for comprehensive strategies to manipulate *TERT* activity for therapeutic purposes.

The interplay between telomerase and ALT pathway presents a complex and dynamic landscape in telomere maintenance, particularly in cancer cells. While telomerase-positive cells maintain telomere length by adding telomeric repeats via the telomerase enzyme, ALT-positive cells use a recombination-mediated mechanism to achieve similar goals [12]. This recombination process may involve the use of telomeric repeats as copy templates, resulting in a heterogeneous telomere length distribution, a hallmark of ALT-dependent cells [57]. The regulation of telomere length by these distinct mechanisms underscores the importance of telomeric repeat RNA (*TERRA*) in telomere maintenance in ALT-positive tumors. *TERRA* has been observed to play a key role by inhibiting telomerase activity in vitro and by directly inter-

acting with telomerase components such as *TERT*, independently of *TERC* [58]. This inhibition of telomerase by *TERRA* suggests a potential natural regulatory mechanism that could influence the activation of the ALT pathway. The dynamics between these pathways are further complicated by the fact that some telomerase-negative cancers resort to the ALT pathway to maintain telomere length, suggesting a potential switching mechanism triggered by telomerase inhibition. Given this interdependence, therapeutic interventions targeting one pathway must consider the potential compensatory activation of the other. Inhibition of telomerase activity alone may inadvertently promote activation of the ALT pathway, thereby sustaining telomere maintenance and tumor cell proliferation. Therefore, a comprehensive understanding of the interactions between telomerase and the ALT pathway is essential for the development of effective anticancer therapies that can disrupt telomere maintenance and limit tumor growth. Other studies indicate that *hTR* upregulation is associated with epigenetic modifications, such as DNA methylation and histone modifications, which can increase telomerase activity. Hypermethylation of the *TERT* promoter is commonly observed in advanced BC, leading to increased gene transcription and increased enzymatic activity. Similarly, *hTR* interaction with regulatory noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), plays a key role in modulating telomerase function. For example, some miRNAs, such as miR-138-5p, miR-100, and miR-1182 [59, 60], have been identified as negative regulators of *TERT* and *hTR*, while their downregulation in BC promotes telomerase activation and tumor progression. Studies have identified several key miRNAs associated with *TERT* regulation in human cancers, as summarized in Table II [61–63].

One of the best characterized is miR-138-5p, which binds directly to the 3′UTR of the TERT mRNA, leading to decreased *TERT* expression. A significant decrease in its levels has been observed in cancers, including BC, which correlates with both increased telomerase activity and invasive characteristics of the tumor. Another negative regulator of TERT is miR-133a. In cancer cells, its expression is reduced, contributing to increased TERT levels and telomerase activation. Consequently, this facilitates uncontrolled cancer cell proliferation [25]. MiR-491-5p exhibits similar properties, also interacting with the 3′UTR of TERT and reducing its levels. Studies have shown that low levels of this miRNA are associated with increased telomerase activity and a more aggressive tumor phenotype [64]. MiR-1182 also directly affects TERT by binding to its coding sequence (ORF), resulting in reduced expression and decreased telomerase activity. In BC models, overexpression of miR-1182 has been shown to inhibit cell proliferation, invasion, and migration, as well as to increase sensitivity to cisplatin [60]. In turn, a group of miRNAs, including miR-1203, miR-1285-5p, and miR-1303, has been associated with mutations in the TERT gene promoter. Their expression is upregulated in some cancers, suggesting a potential role in regulating TERT expression in a promoter-dependent manner, although the precise mechanism remains unclear [63]. MiR-33a-5p and miR-3615 have been observed to be upregulated in the presence of TERT promoter mutations. Their presence cor-

**Table II.** miRNAs associated with *TERT* gene regulation

| miRNA | Role in TERT regulation | Impact on cancer |
|---|---|---|
| miR-138-5p | Directly binds to the 3′UTR of TERT, reducing its expression | Its decrease in cancer increases telomerase activity [25, 61, 62] |
| miR-491-5p | Inhibits TERT expression by interacting with 3′UTR | Reduced levels correlate with telomerase activation, tumor suppressor [62, 64] |
| miR-133a | Targets TERT, reducing its level | Reduced expression is observed in cancers [25, 61, 62] |
| miR-1182 | Directly regulates TERT by binding to ORF | Inhibits proliferation, invasion and metastasis of cancers [60, 62] |
| miR-1203, miR-1285-5p, miR-1303 | Associated with mutations in the TERT promoter | Increased expression in some cancers [63] |
| miR-33a-5p, miR-3615 | Associated with more aggressive cancers. The mechanisms of action are not yet fully understood. | Increased levels in context of TERT promoter mutations, correlate with aggressive cancer phenotype [63] |
| miR-342-5p, miR-541-3p | Directly bind to the 3′UTR of hTERT, reducing its expression and telomerase activity | Reduced proliferative potential of cancer cells and act as tumor suppressors [61, 62] |
| let-7g-3p | May bind to TERT mRNA, affecting its level | Potential effect on cancer proliferation [25, 61, 62] |

relates with a more aggressive tumor phenotype, but their mechanisms of action require further investigation [63]. Other miRNAs, such as miR-342-5p and miR-541-3p, directly bind to the 3′UTR of hTERT. Their presence reduces telomerase activity, while blocking endogenous activity leads to its increase. Therefore, both miRNAs act as TERT suppressors, limiting the proliferative potential of cancer cells [61]. Additionally, the let-7 family, to which miR-let-7g-3p belongs, acts as a tumor suppressor for many oncogenes, including the TERT gene. It has been shown that let-7g-3p can directly bind to the 3′UTR sequence of the TERT mRNA, leading to decreased stability and expression, and consequently reducing telomerase activity and the proliferative potential of cancer cells. This potentially reduces cancer cell proliferation. Moreover, Li *et al*. [65] reported that miR-200a-3p acts as a BC suppressor by inhibiting cell proliferation and increasing apoptosis via STAT4 inhibition. Lower levels are associated with poorer prognosis and may act as a prognostic biomarker. Similarly, Borkowska *et al.* [66] observed that miR-19a-3p and miR-99a-5p correlate with tumor characteristics and may also act as prognostic biomarkers in BC. These data suggest that these miRNAs may be valuable regulatory biomarkers of BC progression and potential therapeutic targets for strategies aimed at suppressing telomerase activity.

Alterations in key oncogenic pathways such as the PI3K/AKT/mTOR and Wnt/β-catenin signaling cascades further drive BC progression by increasing hTR expression and telomerase activity. These pathways regulate cell proliferation, survival, and resistance to apoptosis, ultimately facilitating tumor growth and invasion. Activation of the PI3K/AKT/mTOR pathway contributes to neoplastic transformation by promoting uncontrolled cell cycle progression, enhancing anabolic metabolism, and inhibiting the mechanisms of apoptosis and autophagy that normally limit abnormal cell growth. Akt-dependent phosphorylation (also known as protein kinase B) stabilizes telomerase reverse transcriptase and facilitates its translocation to the nucleus, leading to the maintenance of telomerase activity and cellular immortality [67]. In parallel, dysregulation of the Wnt/β-catenin pathway induces transcription of oncogenes such as c-Myc and cyclin D1, as well as stemness-related genes, which disrupt normal epithelial differentiation and polarity. Nuclear accumulation of β-catenin interacts with TERT to amplify transcriptional programs that promote epithelial-to-mesenchymal transition (EMT) and genomic instability – two fundamental processes driving neoplastic transformation [68].

Remarkably, the interplay between telomerase activation and EMT promotes a more aggressive cancer phenotype, increasing the likelihood of metastasis. Given these observations, targeting hTR and its associated molecular networks represents a promising avenue for therapeutic intervention in BC [69]. This reactivation of telomerase is essential for cancer progression, as it enables cancer cells to maintain their telomere length, thus bypassing the normal cellular growth arrest mechanisms and promoting cell immortalization. Consequently, understanding these regulatory pathways offers potential therapeutic targets for inhibiting telomerase activity in BC cells.

Bladder cancer is a malignancy in which the development of non-invasive methods for diagnosis and disease monitoring is of particular clinical importance. Traditional urine cytology is characterized by high specificity, but its sensitivity is limited, especially in low-grade tumors. In recent decades, numerous tests based on the assessment of telomerase activity have been introduced, including TRAP protocols, ELISA immunoenzymatic assays, and hTERT immunocytochemistry in urinary sediment. The use of these methods has demonstrated the potential to improve the detection of urothelial lesions, but significant barriers remain related to the standardization and reproducibility of results, as well as technical and organizational requirements limiting their wider implementation in clinical practice. Therefore, increasing attention is being paid to genetic and epigenetic biomarkers analyzed in DNA isolated from urine, including TERT gene promoter mutations, which can be detected even many years before clinical manifestation of the disease [70]. Studies using targeted sequencing of a 23-gene panel have shown that most key somatic alterations in BC can be identified in urinary DNA, supporting both diagnosis and assessment of recurrence risk. A subsequent study by Ward et al. (2022), including 884 samples, demonstrated that DNA analysis from urinary sediment achieved high sensitivity (87.3%) and specificity (84.8%) in diagnosing newly diagnosed cases and predicted the risk of recurrence in NMIBC (HR 2.6). These results indicate that mutational sequencing may reduce the need for cystoscopy and provide a valuable tool in oncological surveillance [71, 72].

Telomerase, due to its ubiquitous expression in most cancer cells, represents a promising universal tumor antigen and an attractive target for immunotherapy. Its fundamental role in maintaining cancer cell immortality makes therapeutic targeting of this enzyme an intensively developed anticancer strategy [73]. Inhibition of telomerase activity, thereby inducing replicative senescence and apoptosis in cancer cells, is achieved through several approaches currently in preclinical and clinical trials. Oligonucleotide-based inhibitors are a leading example of telomerase inhibitors. Imetelstat (GRN163L), as a significant representative

of this group, generates significant interest in oncology due to its unique mechanism of action. As a 13-nucleotide, lipophilic oligonucleotide phosphoramidate analogue, it acts as an antisense oligomer complementary to the hTERC, leading to its direct inhibition [74]. Although its clinical development is primarily focused on hematological malignancies, its potential in solid tumor therapies, including BC, should be the subject of intensive preclinical studies. To date, there are no registered or active clinical trials evaluating the efficacy of imetelstat in BC, indicating that its potential use in this disease remains in the early stages of evaluation. However, the results of available clinical trials in other cancers provide justification for further exploration of this therapy in BC, particularly given the high incidence of disorders associated with deregulated telomerase activity in this patient population. Antisense oligonucleotides (ASOs) are designed to selectively bind to telomerase mRNA, leading to its degradation and inhibition of protein synthesis. Studies have shown that delivery of ASOs to BC cells can effectively reduce telomerase activity, leading to telomere shortening and inhibition of tumor growth [75]. A phase 1 clinical trial by Hong *et al*. [76] evaluated MRX34, a microRNA-based anticancer therapy, in patients with advanced solid tumors, including BC. Although the toxicity profile was initially manageable, the study was prematurely terminated due to serious immunological adverse events that led to the death of four patients. Nevertheless, this is the only clinical trial to date that provides proof of concept for miRNAs as a potential therapeutic target in BC.

In the context of immunotherapy, TERT is considered a universal tumor antigen capable of activating an adaptive immune response. T lymphocytes, both CD4+ and CD8+, recognize TERT-derived peptides, leading to tumor growth inhibition. These findings provide a theoretical basis for the development of TERT-based cancer vaccines. Clinical trials using vaccines such as GV1001, UV1, and Gx-301 are already underway in various cancer types. Simultaneously, the development of small-molecule inhibitors is a promising avenue. Research on molecules such as BIBR1532 and imetelstat is already at an advanced stage. BIBR1532 acts by noncompetitively binding to the active site of TERT, inhibiting its enzymatic activity.

Although the mentioned therapeutic solutions are primarily being studied in other cancers, such as pancreatic and lung cancer, their use in BC is under active investigation, paving the way for potential combination therapies.

## Conclusions

Telomerase activation is crucial in BC, enabling uncontrolled proliferation and tumor progression. hTERT upregulation, TERT promoter mutations, epigenetic modifications, and dysregulated miRNAs drive telomerase activity, with key oncogenic pathways such as PI3K/AKT/mTOR and Wnt/β-catenin further enhancing tumor aggressiveness. Differences in telomerase expression between NMIBC and MIBC highlight its potential as a prognostic biomarker. While telomerase remains a promising therapeutic target, further research is needed to develop effective inhibitors, gene-editing strategies, and miRNA-based therapies. Understanding these complex mechanisms of transcriptional and epigenetic regulation of hTERT in BC cells is crucial, as it reveals potential targets for therapeutic interventions. Inhibiting the activity of key transcriptional activators or modulating epigenetic modifications of the hTERT promoter could limit telomerase expression in cancer cells and thus their ability to proliferate uncontrollably. This highlights the need to develop comprehensive therapeutic strategies that target these specific hTERT regulatory mechanisms in BC.

## Ethical approval

Not applicable.

## Conflict of interest

The authors declare no conflict of interest.

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