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Review Article

hTERT, hTR and TERT promoter mutations as markers for urological cancers detection: A systematic review

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Abstract

The clinical relevance of telomerase subunits (human reverse transcriptase − hTERT, and human telomerase RNA − hTR) and TERT promotor mutations as biomarkers in genitourinary cancers was reviewed through the systematic analysis of the current literature.

We performed a systematic literature search using 2 databases (Medline and Scopus) over the past 20 years. Primary outcomes were sensitivity and specificity of hTR, hTERT and TERT promoter mutations. Secondary outcomes were the biomarkers predictive values for tumor characteristics.

Regarding bladder cancer, hTERT in urine showed high sensitivity (mean values: 55%−96%), and specificity (69%−100%): it correlated with bladder cancer grade and/or stage. hTR sensitivity ranged from 77% to 92%. With adapted cut-off, it demonstrated 72% to 89% specificity. TERT promoter mutation rate was up to 80% both in tissue and urine, resulting in 62%-92% sensitivity for primary tumors and 42% for relapse. Specificity ranged from 73% to 96%, no correlations with stage were observed. In prostate cancer, hTERT in tissue, prostate secretion and serum showed high sensitivity (97.9%, 36%, and 79.2%−97.5%, respectively) and specificity values (70%, 66%, 60% −100%). hTR showed very high sensitivity (88% in serum and 100% in tissue) although specificity values were highly variable depending on the series and techniques (0%−96.5%). In RCC, hTERT sensitivity on tissue ranged from 90 to 97%, specificity from 25 to 58%. There was an association of hTERT expression with tumor stage and grade.

hTERT showed high accuracy in genitourinary cancers, while the value of hTR was more controversial. hTERT and TERT promotor mutations may have predictive value for bladder cancer and RCC staging and grading, while no such relationship was observed in CaP. Although telomerase subunits showed clinically relevant values in genitourinary cancers, developing fast and cost-effective methods is required before contemplating routine use.

Keywords: Systematic review; Bladder cancer; Renal cell carcinoma; Prostate cancer; Htert; hTR; TERT promoter mutations

1. Introduction

Bladder cancer (BCa) diagnostic and follow-up rely on imaging and cystoscopy with biopsy or urine cytology, prostate cancer (CaP) on imaging and image-guided biopsy, renal cell carcinoma (RCC) on imaging alone. All show limitations as invasive methods are ill-adapted to population screening [1] while noninvasive methods may lack sensitivity or specificity and cannot be usually relied upon to anticipate tumor grade and stage [2−5].

Urine cytology [6] is a well-established method in high-grade BCa detection although it shows high specificity but poor sensitivity in low grade tumors. Other biomarkers

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include orosomucoid 1, survivin, eukaryotic initiation factor 2, nuclear matrix protein, apolipoprotein A-1, calprotectin, cytokeratins, ubiquitin 2, select miRNAs and more than 30 other markers used alone or in combination [7]. PSA is a well-known marker for CaP screening and follow-up, shows significant limitations [8] as it is not disease-specific but organ-specific. No biomarkers are to date available for RCC.

To circumvent these limitations, efforts are made to develop specific biomarkers in cancer detection and risk-stratification. From initiation to promotion and progression, most solid malignancies undergo successive genetic changes that must be passed to their descendent to fulfill the unrestricted development that characterizes cancer growth [9]. While biomarkers in tissue have a limited application predominantly for laboratory diagnostic with immunohistochemistry or research, the detection of them in urine or blood would have a clinical use.

Because DNA polymerases are unable to replicate the 5' end of linear DNA [9] each mitosis entails a minute loss of DNA and a shortening of chromosomes. Telomeres that are repetitive TTAGGG sequences at the distal end of the DNA helix, protect the DNA coding segments from being truncated at division at the expense of their own shortening. They also contribute to chromosomal integrity in eukaryotic cells. Although subject to restoration by a complex formed by the non-coding RNA (hTR) that their DNA sequence encodes and the human telomerase reverse transcriptase (hTERT), telomeres overall shorten with age. When they reach a critical length, the cell is driven to programmed death [11,12]. As illustrated in Fig. 1, telomerase activity is a Janus-faced biomarker that is implicated in the process of senescence when it fails to counteract the lifelong shortening of telomeres and in cancer when it unrestrictedly sustains the division of cancer cells.

Intriguingly, in spite of the strong literature on the mechanisms and consequences of telomerase activity in human cancers [10], and of the wide availability of the techniques to analyze hTERT the mutations of its promoter (TERT) and hTR, this field is still awaiting clinical applications. There is a number of original studies and reviews addressing the role of telomerase and its subunits in urological cancer detection and follow-up. These biomarkers have been well-known for a rather long time, but they are not routinely used, and their exact application is still not clear. Based on this, one may suppose they are unsuitable for clinical use, but the articles report high accuracy of these biomarkers. Besides, to our best knowledge, each of the articles focuses on 1 disease or 1 marker (e.g., telomerase activity, or mutation presence). To contribute to the reappraisal of telomere biology in urology, we conducted a systematic analysis of biomarkers applications in BCa, RCC, and CaP. This review intends to summarize these findings comprehensively, to "draw the line" under the available data on these biomarkers and define which marker has diagnostic and prognostic value for which cancer.

2. Materials and methods

2.1. Search strategy, inclusion criteria

The present systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (see PRISMA statements, **Fig. 1**). The detailed search strategy and review protocol has been published in Prospero (ID 178460). The scope of the review according to PICO process (Patient, Intervention, Comparison, Outcomes) is as follows:

P − patients with common urological cancers (bladder cancer, renal cell carcinoma, CaP)

I − detection of hTERT (human telomerase reverse transcriptase mRNA) / hTR (human telomerase RNA) and TERT promoter mutations in urine OR in tissue

C − urine cytology OR histology

O − sensitivity and specificity of hTERT/hTR/TERT promoter mutations

We performed a systematic literature search using 2 databases (Medline (PubMed) and Scopus) over the past 20 years with the following search terms: telomerase AND (bladder cancer OR urothelial cancer), telomerase AND ((kidney cancer) OR (kidney tumor)), telomerase AND CaP. "hTERT, hTR, TERT promotor" were not included in the search as all articles on these subunits also included the term "telomerase". Two authors (AS and NP) independently reviewed headings and abstracts to exclude non relevant publications such as reviews, comments, papers in languages other than English, and articles which dealt with other biomarkers or with conditions other than listed (bladder adenocarcinoma, Wilms tumor, etc.). In the event of disagreement between the reviewers, articles were retained for the following step of selection.

After *in extenso* review of the publication, the 2 readers (AS, NP) excluded those where the authors focused on laboratory techniques without clinical data. In the event of disagreement AS and NP sought to justify their decision and tried to resolve the disagreement. If they failed to reach an agreement, a senior researcher (AM) made the final decision. Our systematic review includes all original articles containing data on hTERT/hTR/TERT promoter mutations in urothelial cancer, renal cell carcinoma and CaP.

2.2. Data extraction outcomes

The following raw data was extracted manually from the articles: number of treated patients, tumor stage and grade, methodology of biomarkers measuring, outcomes and diagnostic performances.

The primary outcomes were sensitivity and specificity of hTR, Htert, and TERT promoter mutations as markers for cancer detection and follow-up.

The secondary outcomes were the biomarkers predictive value for tumor characteristics and association between diagnostic accuracy and methodology used for biomarkers

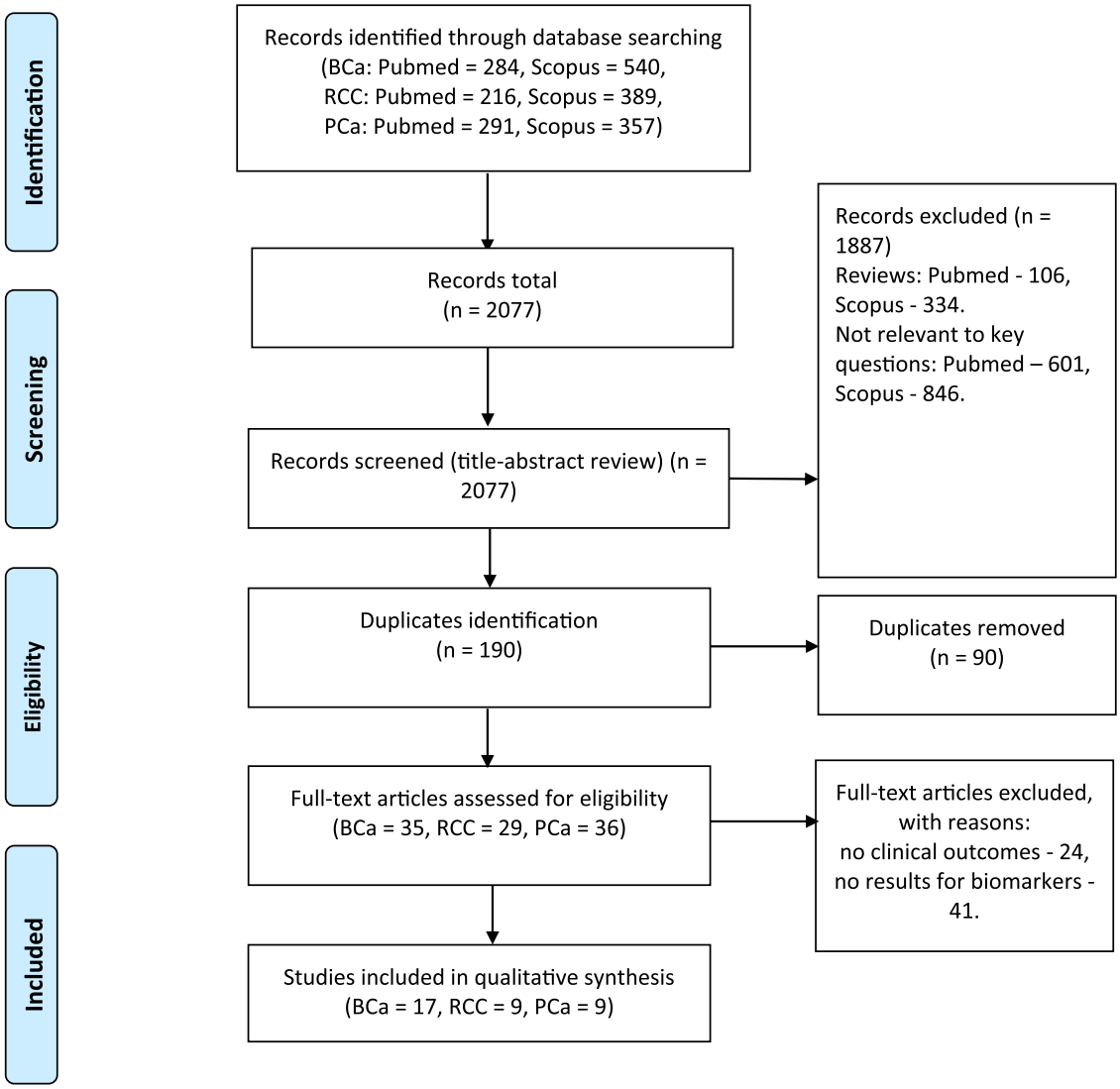

Fig. 1. PRISMA statement.

detecting. We analyzed the association between biomarkers expression and cancer stage and grade, lesion size and quantity (for bladder and kidney).

Due to the high heterogeneity in the studies with regards to methodology and the conditions in control groups, it was not possible to perform a meta-analysis. Thus, the authors conducted a qualitative narrative synthesis of the obtained data.

3. Results

After applying all the selection criteria, the final sample included 17 articles related to BCa [9,13–28]. Nine of them dealt exclusively with the detection of hTR, hTERT and/or TERT promoter mutations in urine [13,16,18,22–27], 6 dealt exclusively with its detection in tissue [9,14,15,17,19,20], and 2 articles [21, 28] dealt with both urine and tissue. Nine articles related to RCC [29–37] and 12 to CaP [38–49]. All studies on BCa, and the majority of studies of RCC and CaP were prospective. No connections between telomerase subunits expression and features not related to cancer, such as age or gender, were found.

3.1. BCa

The most common telomerase subunit assessed was hTERT (**Table 1**). In urine eight studies compared hTERT expression in cancer and control groups [13,18,21–25,27], most also included cytology results. The common methodology to quantify hTERT was reverse transcriptase polymerase chain reaction (RT-PCR) in voided second morning urine. However, hTERT could also be analyzed in wash-out cytology after cystoscopy [13,24], or by immunochemistry of urinary sediments [22].

Neves et al. [25], Weikert et al. [27], Eissa et al. [18], and Mezzasoma et al. [24] reported significant positive correlation between hTERT expression and BCa grade and/or stage, but no correlation between hTERT and the size or

Table 1
Bladder cancer (BCa), telomerase in urine.

Author, year	Number of patients	Telomerase subunits in urine/bladder washings	Cytology results	Sensitivity	Specificity
Fukui et al. 2001, Molecular Urology [13]	35 patients - bladder cancer group 14 patients - follow-up group (no BC at present, had BC in the past) 14 patients - noncancer group	The expression of hTERT mRNA (cut-off value 0.27%): Cancer group - 30/35 (86%) Follow-up group - 2/14 (14%) Noncancer group - 1/14 (7%) Positive correlation only with tumor size >3 cm. G1 - 5/7 (71%) G2 - 18/23 (78%) G3 - 3/5 (60%)	G1 – 1/7 (14%) G2 – 15/23 (65%) G3 – 4/5 (80%) Lower sensitivity then hTERT, except for G3.	85.7%	89.3%
Neves et al. 2002, The Journal Of Urology [25]	50 patients - BC 50 patients - control group (no history of cancer, normal cystoscopy)	hTERT in: cancer - 37/50 (74%) control - 12/41 (29%)	Positive: G1 - 0% G2 - 25% G3 - 55%. Sensitivity 31% (18-48), specificity 100% (89-100)	75% (61-86), increased with decease stage	69% (53-82)
Melissourgos et al., 2003, Basic Science [23]	146 patients - bladder cancer (132 TCC, 14 other malignancies) 128 patients - control group	In cancer group total 134 (91.8%) In situ – 10 (83%) Ta (G1) – 36 (92%) T1 (G1) – 13 (93%) Ta (G2) – 22 (88%) T1 (G2) – 17 (89%) T1 (G3) – 13 (100%) T2 (G3) – 7 (88%) T4 (G3) – 2 (100%)	Positive: In situ – 4 (33%) Ta (G1) – 11 (28%) T1 (G1) – 4 (29%) Ta (G2) – 12 (48%) T1 (G2) – 9 (47%) T1 (G3) – 8 (62%) T2 (G3) – 5 (63%) T4 (G3) – 2 (100%)	91.8%. Positive predictive value 96.4%.	96.1%. Negative predictive value 91.1%.
Weikert et al., 2005, Int. J. Cancer [27]	179 patients - BC 186 patients – benign urological conditions (control group) 100 healthy individuals (control group)	hTR (cutoff value of 800 yields): Ta - 42 (61.8%) T1 - 23 (87.9%) T2 - 22 (88%) T3 - 12 (100%) T4 - 4 (100%) Tis - 4 (80%) hTERT (cutoff value near the detection limit): Ta - 29/71 (40.8%) T1 - 25/39 (64.1%) T2 - 18/29 (62.1%) T3 - 10/14 (71.4%) T4 - 4/4 (100%) Tis - 3/5 (60%).	Positive: Ta – 15/74 T1 - 18/37 T2 - 13/31 T3 - 6/14 T4 - 2/3 Tis - 3/5	hTR - 77% hTERT - 55.2% Increased with decease stage and grade. Combined hTR and hTERT detection had no significant advantage over hTR detection alone	hTR - 72.1% hTERT - 85.0% Increased with stage and grade decrease.

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Khalbus and Goodison, 2006, CytoJournal [22]	29 – malignant cases (group 1) (28.7%) 39 – non-malignant cases (group 2) (38.6%) 33 – cases of cytological atypia (group 3) (32.7%)	hTERT expression: Group 1: 27/29 (93.1%) Group 2: 3/39 (7.7%)	Positive in malignant: 29 (88%). Negative in benign: 11 (43%).	84.8%. Positive predictive value 77.8%	65.2%. Negative predictive value of 75%
Eissa et al., 2007, The journal of urology [18]	200 patients - bladder carcinoma 85 patients - benign bladder lesions 30 - healthy individuals (control group)	Positive: 1) **RTA** -stage: early – 20 (59%), late - 130 (78%); -grade: 1-2 - 60 (73%), 3 - 90 (76%). 2) **hTR** -stage: early - 30 (88%), late - 154 (93%); -grade: 1-2 - 80 (98%), 3 - 104 (88%). 3) **hTERT** -stage: early - 26 (77%), late - 166 (100%); -grade: 1-2 - 82 (100%), 3 - 110 (93%).	-stage: early - 24 (71%), late - 126 (76%); -grade: 1-2 – 56 (68%), 3 -94 (80%).	1) RTA: 75% 2) hTR: 92% 3) hTERT: 96%	1) RTA: 92% 2) hTR: 89% 3) hTERT: 96%
Mezzasoma et al., 2010, BMC Urology [24]	36 patients - BC 58 patients - control group	1) Superficial BC hTR (median): 7.03×10^{-4} hTERT (median): 2×10^{-5} 2) Invasive BC hTR (median): 2.6 × 10-4 hTERT (median): 1.1×10^{-4}	–	AUC 0.72 (95% CI: 0.62-0.83) for hTR, 0.76 (95% CI: 0.65-0.87) for hTERT. Positive correlation with stage.	-
Papadopoulos et al., 2013, Cancer Research [26]	1) 76 noninvasive papillary urothelial carcinomas and flat carcinoma in situ (tissue) 2) 14 patients with follow-up cystoscopy for non-muscle-invasive urothelial carcinoma (urine).	TERT promoter mutations: (1) Group 1: 56/76 (74%) + no correlation between grade and type (2) Group 2: 11/14 (79%)	–	–	–
Glukhov et al., 2014, Biomedical Chemistry [21]	20 patients with BC 10 patients with cystitis - control group	hTERT expression: 16 (80%) in BC vs 0 in control hTR expression: 20 (100%) in BC vs 10 (100%) in control. No correlation between stage and grade	–	hTERT: 80% hTR: 100%	hTERT: 100% hTR: 0%
Allory et al., 2014, European urology [28]	A group of 135 patients with urine samples from 468 patients in 3 countries for TERT in tissue assessment	–	–	TERT: 62% for primary tumor, 42% for relapse	TERT: 73-90%
Descotes et al., 2017, British Journal of Cancer [16]	348 patients - UBC (280 mutated, 68 wild type) 167 patients - control group (89 healthy individuals, 17 neurogenic bladder, 10 infectious urines, 42 patients with any other type of cancer)	TERT mutation rate 80.5% in UBC group No correlation with stage, positive correlation with grade.	Negative - 115 Atypical urothelial cells of undetermined significance - 19 Low-grade - 97 AUC-H (cannot exclude high grade) - 8 High-grade - 109	Low-grade pTa: 74.3% High-grade pTa/pTis - 92.5% pT1 - 77.6%	92% in control group, with 96% in inflammation and 88% in CaP

number of lesions. In contrast, Melissourgos et al. [23] and Glukhov et al. [21] observed no correlations between hTERT, tumor stage or grade (**Table 1**). Weikert et al. [27] and Eissa et al. [18] who carried out quantitative PCR, also failed to identify links between increased hTERT and cancer detection. Overall, hTERT showed clinically relevant figures for BCa detection in terms of sensitivity (55%−96%) and specificity (69%−100%). hTR was researched by RT-PCR in urine in 4 reports [18,21,24,27]. While all showed high sensitivity values (77%−92%) specificity results were controversial. Glukhov et al. [21] detected hTR in all participants, irrespective of the presence of cancer, which amounted to a complete lack of specificity (0%). Intriguingly with the same methods others reported high specificity (72%−89%) and a positive correlation with stage and grade [18,27].

Three reports analyzed TERT promoter mutation in urine [16,26,28]. High prevalence of mutations supported 62% to 92% sensitivity for primary tumors and 42% for relapse while specificity varied from 73% to 96%. No correlation with stage was observed, although Descotes et al. [16] observed a higher rate of mutations in high grade cancers, compared to well-differentiated cancers (85% and. 74%, respectively, $P = 0.015$). All reports stated that cytology results correlated with BCa stage and grade, but had disparately lower accuracy than hTERT and hTR, except for G3 cancer [13].

Two studies assessed hTERT and hTR in tissue samples [20,21] (**Table 2**). Takibana et al. reported a positive correlation between hTERT concentration and tumor stage, but no correlation with tumor grade. Conversely, positive correlation between hTR and tumor grade, but no correlations with stage, were observed. Glukhov et al. found no correlation of these markers with both stage and grade.

Last, 4 series detailed the mutations of TERT promoter in tissue [9,14,17,28]. All reported a strong association of BCa presence and mutation rate (up to 77%) although no relationships with grade, stage, size, or tumor quantity were demonstrated.

3.2. Renal cell carcinoma

Of nine articles on RCC, seven used RT-PCR to quantify hTERT (**Table 3**). Three authors [30,33,34] compared hTERT expression in tumor and adjacent normal renal tissue to evaluate its diagnostic accuracy. Sensitivity varied from 90% to 97%, specificity from 25% to 58%. Fan et al. [30] emphasized, that different hTERT transcripts were also present in normal cells, although they did not entail higher telomerase activity. They suggested that only full-length hTERT transcripts characterized RCC, which in their hands showed 100% specificity and 82% sensitivity.

Zanjani et al. [35], performed immunohistochemistry (while the rest of authors used RT-PCR) to demonstrate variations in sensitivity according to the histology type (clear cell RCC: 57.5%, papillary RCC: 65.6%, chromophobe RCC: 0%). In ccRCC, significant associations between hTERT expression and stage, grade, tumor size, microvascular invasion, lymph node invasion, renal pelvis and sinus fat involvement, Gerota's fascia invasion, distant metastasis were highlighted. In contrast, Martino et al. [29] observed positive correlation of hTERT with stage (T3/4 vs. T1/2: 56.6 vs. 22.1 ng/mL, $P = 0.037$) and metastatic status (M1 vs. M0: 91.7 vs. 27.5 ng/mL, $P = 0.003$), but no association with N stage ($P = 0.71$) and grade ($P = 0.64$). Sitaram et al. [36] found no difference in hTERT expression depending on grade or stage, and, of pivotal importance, no impact on survival.

Rohde et al. [34] complemented hTERT analysis by measuring hTR expression with in situ Hybridization. However, it was detected in all tissue samples from tumor and kidney parenchyma, corresponding to 100% sensitivity and 0% specificity.

Wang et al. [37] and Hosen et al. [31] observed TERT promotor mutations only in a minority (6.4%−9%) of RCC where these mutations carried prognostic value as they were associated with aggressive ccRCCs (presence of metastases or invasion to the tumor pseudo-capsule), and shorter survival, HR=2.90 (95% CI=1.13−7.39, $P = 0.03$).

3.3. CaP

Among the 12 articles on CaP (**Table 4**), seven used RT-PCR in tissue to demonstrate high sensitivity (46.1−97.8%) and specificity (60%−96.5%) of hTERT. Interestingly, hTERT was also detected by RT-PCR in prostatic secretions and serum [41,43,44,46,47] and was increased in CaP, compared to BPH [43] but also to prostatitis [43,44]. Four reports used immunochemistry, Bettendorf et al. [40] and Atasoy et al. [42] found no significant difference in hTERT expression between benign and malignant tissue, and no association with Gleason grade and tumour staging. In contrast, Gasinska et al. [45,48] observed a negative correlation between hTERT immunostaining and Gleason score. hTR was assessed by RT-PCR in 3 reports where it was readily detected in all samples (100% sensitivity) [46] but with strong variations in specificity as it was detected by some in all benign glands (0% specificity) [46] while others reported almost perfect specificity (96.5%) [47]. Using radioactive in situ hybridization Bettendorf et al. [40] observed more copies in cancer (>20 grains/nucleus in cancer cells, 5−40 grains in preneoplastic intraepithelial lesions and 2−10 grains in normal glands). Last, the sole investigator who assessed TERT promoter mutations in 167 archival specimen found no mutations [38].

4. Discussion

Contrary to the emphasis following the 2009 Nobel Prize in Medicine on telomerase in cancer, no solid paradigm emerged from the systematic review of the literature on telomeres in urologic malignancies. In view of such a

Table 2
BCa, telomerase in tissue.

Author, year	Number of patients	Telomerase subunits/their mutations in tissue	Sensitivity	Specificity
Takihana et al., 2006, International Journal of Urology [20]	29 patients - superficial and advanced BCs	**hTERT:** hTERT/GAPDH mRNA 5.13 ± 1.21 in T2-T4 *vs* 0.45 ± 0.14 in Ta-T1, $P = 0.001$ hTERT mRNA/total RNA [32.64 ± 10.09] × 10^9 copies/μg in T2-T4 *vs.* [8.24 ± 1.50] × 10^9 copies/μg in Ta-T1, $P = 0.0028$. Positive correlation with tumor stage, no significant connection with grade. **hTR:** hTR/GAPDH mRNA 3.36 ± 2.15 in T2-T4 vs 19.28 ± 6.10 in Ta-T1, p=0.0012 hTR mRNA/total RNA [38.99± 15.68] × ∼10^9 copies numbers/μg in T2-T4 vs. [142.05± 59.59] × ∼10^9 copies numbers/μg in Ta-T1, $P = 0.0091$. Positive correlation with tumor grade.	–	–
Glukhov et al., 2014, Biomedical Chemistry [21]	20 patients with BC	hTERT expression: 16/20 (80%) hTR expression: 20/20 + no correlation between stage and grade	80%	100%
Allory et al., 2014, European urology [28]	468 patients in 3 countries	TERT mutation present at 77% cases. No association with age, sex, or smoking. The frequency is similar in low-risk NMIBC (73%), high-risk NMIBC (74%), and in MIBC (53%) (p = 0.192), and in newly diagnosed versus recurrent tumors	–	–
Hosen et al., 2015, International Journal of Cancer [17]	327 patients with BC	Overall TERT promoter mutations 65.4% Low-grade tumors - 73.7% High grade tumors - 63.3% Non-invasive tumors - 66.3% Invasive tumors - 68.4%	–	–
Wang et al., 2015, The Oncologist [9]	185 patients with UBC	hTERT mRNA expression Mutated - 27 Wild type − 16 No correlation with patient age, tumor size and quantity, TNM stage, grade.	52%	95%
Cheng et al., 2016, Histopathology [14]	26 - classic inverted papilloma 26 - urothelial carcinoma with inverted growth 71 - conventional urothelial carcinoma 25 cases of cystitis glandularis from non-neoplastic bladder	TERT promoter mutation (1) inverted papilloma - 4 (15%) (2) urothelial carcinoma with inverted growth - 15 (58%) (3) conventional urothelial carcinoma - 45 (63%) (in pTa and pT2 23 (64%) vs. 22 (63%)) (4) cystitis glandularis - 0‘	–	–
Roggisch et al., 2019, Urologic Oncology [19]	75 patients	TERT promotor mutations: Low grade: 21 (33.3%) High grade: 50 (66.7%) Ta - 25 (40%), T1 - 18 (29%), T2 − 17 (27%)	Invasive BC: 80% Noninvasive BC: 86%	–
Kurtis et al., 2016, Annals of Diagnostic Pathology [15]	86 specimens	64 (74%) carried 1 of the 2 mutations: C228T - 54 (84.4%) C250T - 10 (15.6%) No correlation between grade, invasiveness, UG tract (upper or lower).	–	–

strong scientific rationale, the discrepancies observed in the urology literature might result from the variable techniques used to approach telomeres biology and from the specifics of the diverse materials in which they were researched.

In keeping with its canonic role in healthy tissues, hTR was readily detected in prostate, bladder and kidney non-cancer tissues, although at variable concentrations. hTERT regulates the enzyme activity and normally its

Table 3
Renal cell carcinoma, telomerase.

Author, year	Number of patients	Telomerase subunits/their mutations	Sensitivity	Specificity
Rohde et al., 2000, Clinical Cancer Research [34]	20	Kidney tissue: hTR − 20 (100%), hTERT − 15 (75%). Tumor tissue: hTR − 20 (100%), hTERT − 18 (90%)	hTR − 100%, hTERT − 90%	hTR − 0%, hTERT − 25%
Paradis et al., 2001, Journal of Pathology [33]	41	hTERT: tumor − 38 (93%). Adjacent tissue − 29 (71%). hTERT mRNA level in tumor *vs* normal tissue = 968.6±80 *vs* 41±4.3, $P = 0.01$. 30 (73%) tumors "hTERT-positive" (expression > baseline in tissue)	hTERT − 93%	hTERT − 29%
Fan et al., 2005, Human Cancer Biology [30]	33	Overall hTERT: tumor − 32 (97%), kidney − 14 (42%). Full-length hTERT: tumor − 27 (82%), kidney − 0.	Overall hTERT − 97%, full-length hTERT − 82%	Overall hTERT − 58%, full-length hTERT − 100%
Sitaram et al., 2009, International Journal of Cancer [36]	176	hTERT expression levels higher in both ccRCC and pRCC comparing to kidney cortex, $P<0.001$ and $P = 0.011$, respectively. No difference between ccRCC and pRCC. No difference depending on grade or stage. No impact on survival.	–	–
Wang et al., 2014, Oncotarget [37]	109 with RCC, 14 patients with UTUC	hTERT level in mutated *vs* wild type RCC = 4.55 ± 3.53 *vs.* 0.115 ± 0.08 $P = 0.0036$) TERT promoter mutations are associated with aggressive ccRCCs (mts or capsular invasion)	TERT promotor mutation in RCC − 9%, in UTUC − 29%.	–
Hosen et al., 2015, International Journal of Cancer [31]	188	Mutations more often in T1/2 than in T3/4, OR=0.15 (95% CI = 0.03−0.72, P= 0.02). No correlation with stage, OR=0.78 (95% CI=0.24−2.56, P= 0.68). Shorter survival in mutation, HR=2.90 (95% CI=1.13−7.39, P= 0.03).	6.4%	–
Martino et al., 2016, Molecular Carcinogenesis [29]	243 with clear cell RCC, 420 age- and gender-matched control	hTERT serum level is similar between cases and controls (P= 0.50). Positive correlation with stage (T3/4 *vs.* T1/2: 56.6 *vs* 22.1 ng/mL, P= 0.037) and mts. (M1 *vs.* M0: 91.7 *vs.* 27.5 ng/mL, P= 0.003). No association with N stage (P= 0.71) and grade (P= 0.64).	–	–
Pal et al., 2017, Urologic Oncology: Seminars and Original Investigations [32]	96	Higher level of hTERT in RCC compared with renal parenchyma (P= 0.04) and in high grade RCC compared with low grade.	–	–
Zanjani et al., 2018, Anatomical Pathology [35]	176	hTERT low-intensity staining 90 (51%), high − 86 (49%). Significant association between hTERT expression and stage, grade, tumor size, microvascular invasion, lymph node invasion, renal pelvis and sinus fat involvement, Gerota's fascia invasion, distant metastasis.	ccRCC − 57.5%, pRCC − 65.6%, crRCC − 0%	–

expression is high in germ and stem cells. In normal cells during DNA replications, telomeres shorten. Critically short telomeres length is a signal which starts apoptosis (**Fig. 2**). Telomerase activity in normal cells is usually suppressed at the transcriptional level. But in the case of mutation in TERT gene promotor, active transcription may start [50].

In BCa, low concentrations of hTR were detected in all urothelial cells, irrespective of their cancer status explaining the dissonance observed in several reports. Glukhov

Table 4
Prostate cancer, telomerase.

Author, year	Number of patients	Telomerase subunits/mutations	Sensitivity	Specificity
Bettendorf et al., 2003, The Prostate [40]	12 − prostate adenocarcinomas	**BPH:** hTR − 2−10 grains/ nucleus, hTERT − weak staining in 40% cells. **PIN:** hTR − 5−40 grains/ nucleus, hTERT − staining in 10%−60% cells, from weak to strong **CaP:** hTR > 20 grains/ nucleus, hTERT − staining in 10%−60% cells No association between Gleason grade, tumour staging, and intensity of telomerase expression	−	−
Kamradt et al., 2003, Laboratory Investigation [46]	46 - prostate tumors 10 - patients with BPH	**CaP:** hTERT mRNA: 45/46 hTR expression: 26/26 Median values of normalized: - hTERT mRNA = 0.04 (0−21.11) - hTR = 34.19 (0.05−3203.35) **BPH:** hTERT expression: 3/10 hTR expression: 10/10 Median value of hTERT mRNA = 0.0063 (0 − 0.0098)	hTERT − 97.8% hTR − 100%	hTERT − 70% hTR − 0%
Crocitto, 2004, Urology [41]	147 men	−	36% (in prostatic secretion)	66% (in prostatic secretion)
Dasi et al., 2006, Annals New York Academy Of Sciences [44]	68 - patients with elevated PSA (26 − CaP, 35 − prostatitis, 7 − non-malignant prostate diseases) 44 patients − healthy volunteers (control group)	Median values for hTERT expression CaP group: (0.72; range 0.01-12.86) Prostatitis group: (0.29; range 0.01-66.07) Control group: (0.13; range 0.02 −0.35)	81%	60%
Atasoy, 2008, International Urology and Nephrology [42]	70 - prostate adenocarcinomas 29 - benign prostate hyperplasias 19 - prostatic intraepithelial neoplasias (PIN)	Nuclear immunoreactivity for hTERT Adenocarcinoma group: 59/70 (84.2%) Hyperplasia group: 24/29 (82.7%) PIN group: 18/19 (94.7%)	−	−
Carbonare, 2010, Urologic Oncology [47]	26 - patients with CaP	hTR expression: 23/26 (88.5%); RNA = 15.34 (0.2−49) pg/ml. hTERT expression: 12/26 (46.2%); RNA = 3.3 (0.8−5.1) pg/ml.	hTR − 88% hTERT − 46.1%	hTR − 96.5% hTERT − 100%
Sabaliauskaite, 2012, Genes, chromosomes & cancer [49]	158 - samples with prostate adenocarcinomas 21 - noncancerous prostatic tissues +65 urine sediments	Expression of hTERT: 75/158 (47.5%) tumors Expression of the hTR transcript: 156/158 (98.7%) tumors hTERT transcript: 11/65 (16.9%) urine sediments; 19/61(31%) specimens were positive for hTERT	−	−
March-Villalba, 2012, Expert Opinion on Biological Therapy [39]	37 − patients with localized CaP 12 − patients with locally advanced CaP	Plasma hTERT mRNA levels Localized: 1.80 (median), 0.21 - 12.0 (range). Locally advanced: 7.4 (median), 1.4 - 13.0 (range).	83%	87%

(*continued*)

March-Villalba, 2012, Plos one [43]	105 - patients with elevated PSA levels (46 CaP, 47 prostatitis, 12 BPH) 68 - healthy volunteers (control group)	Pre-operative hTERT mRNA CaP: 1.6 (0.7−4.08) Prostatitis: 0.13 (0.03−0.31) BPH: 0.03 (0.01−0.13) Control group: 0.07 (0.02−0.10)	91.3% (79.2−97.5)	84.7% (73.0 −92.8)
Gasinska, 2013, Folia histochemica original study et cytobiologica [45]	140 - low advanced PC specimens from patients (after radical prostatectomy)	Nuclear reactivity of hTERT: 81%. Mean LI_{TERT} (labeling index) = 18.0± 1.5%. Decrease of TERT expression with tumor grade (P= 0.025). Mean LI $_{hTERT}$ percentages: 21.1%, 16.1% and 7.3% for low to high-grade groups, respectively. No correlation between number of positively staining hTERT cells and Gleason score (P = 0.142) and pathological tumor stage (P = 0.311).	–	–
Stoehr, 2015, Pathobiology [38]	167 - unselected, archival prostate tumors	TERT core promoter mutation: 0/167 (all cases exhibiting a wild-type sequence)	–	–
Gasinska, 2019, Pathology & Oncology Research [48]	130 - men with clinically localized CaP (after radical prostatectomy)	BR-positive: 14.3 ± 2.3%; BR-negative: 20.3 ± 2.0%. hTERT (P = 0.033) was negatively correlated with the Gleason score. AUC=0.615 (sensitivity 73.0% and specificity 46.3%). LI_{TERT}≤6.7% - negative prognostic factors for BRFS.	–	–

et al. [21] used qualitative PCR to assess hTR. However, the high accuracy of this method resulted in the detection of hTR in all groups of patients; cancer, control and cystitis alike that is a disappointing 0% specificity. Other authors [18,27] used quantitative PCR allowing the use of ROC-analysis to refine the cut-off values for detection. This approach resulted in up to 89% hTR specificity. Summing up, a proper cut-off point is critical for hTR assessment.

Paradis et al. [33] reported the same tendency for hTERT; hTERT level was higher than threshold of quantification in 38/41 tumors (93%) and in 29/41 normal-looking adjacent tissue samples (71%). The authors considered that more than the detection of hTERT, it was its relative increase compared to normal tissue that showed diagnostic value.

In 1994, Kim et al. introduced the highly sensitive isotope Telomeric Repeat Amplification Protocol (TRAP) in

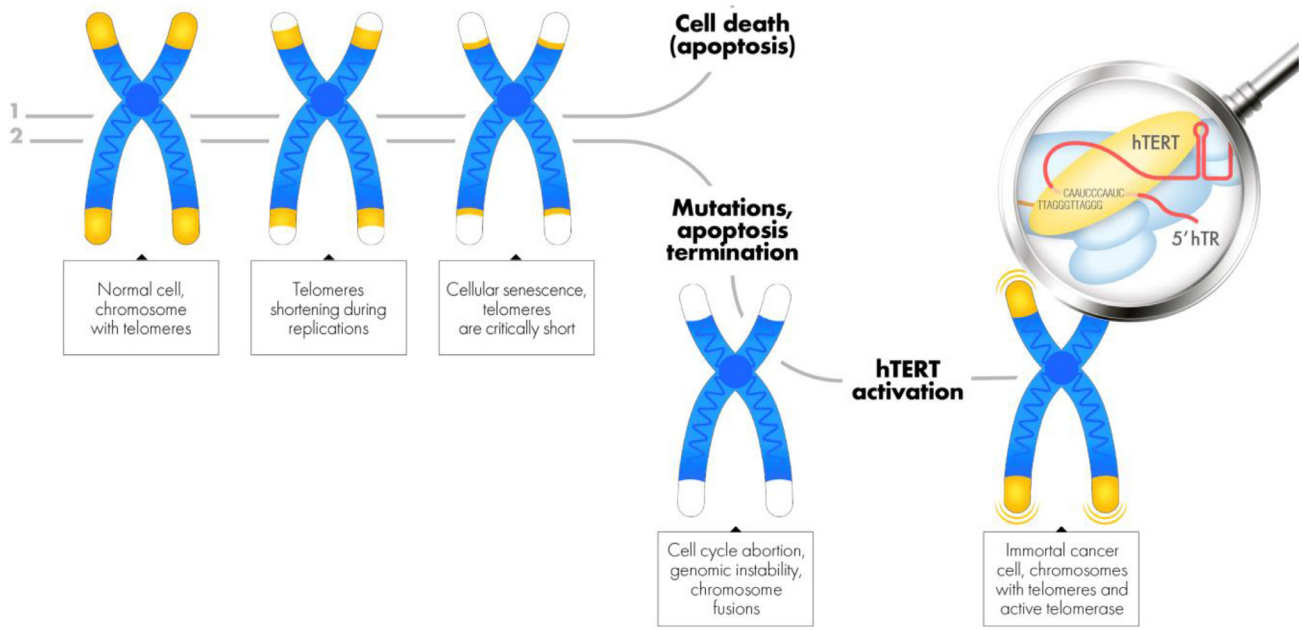

Fig. 2. Telomeres in normal and cancer cell.

the measurement of telomerase activity (TA) and of the concentration of its subunits [51]. However, this method requires radioisotope-labeled nucleotides that are somewhat costly and ill-adapted to the clinical routine [52−54]

Recently, new methods slightly less accurate than TRAP, but more cost-effective were developed, albeit they still require specific reagents and equipment making the detection of TA highly dependent on the locally available facilities [55,56]. Isotope labelling may be replaced by non-radioactive labeling such as in scintillation proximity assay, magnetic particles extraction, fluorescent analysis, TRAP-ELISA, transcription amplification, etc. [57,58]. Some refinements were reported to allow single-cell measurement of TA [59]. During the last 10-15 years, polymerase chain reaction (PCR) became a new standard as it is a faster and cheaper method. In our review, only Khalbuss et al. [22] used TRAP for hTERT detection and Cheng et al. [14] applied high-resolution fluorescence using a melting curve analysis for TERT promotor mutation assessment. Eissa et al. [18] compared TRAP and PCR and confirmed that they were highly correlated although in terms of cost-effectiveness, RT-PCR assessment of hTR was superior. Melissourgos et al. [23] also pointed to the higher cost-effectiveness of PCR.

The present systematic review of the literature of the past 20 years showed that the detection in urine of telomerase subunits (hTERT and hTR) was not only a strong predictor of the presence of BCa but also correlated to tumor stage and grade. Unfortunately, no cost effectiveness analysis was available to evaluate the clinical utility of hTERT and hTR in BCa detection or surveillance. Nowadays, the most common diagnostic tool for BCa is urine cytology: it lends itself well to screening, is relatively fast and cheap [60]. But, as our review showed, the metrics of hTERT and hTR were superior. The main limitations of these methods are expensive equipment and amount of time needed for analysis. While cytology assessment implies in fact simple microscopy, PCR for telomerase subunits is a long complex multi-stage process, which cannot be totally automated. It includes RNA extraction and purification, PCR itself, dilution and measurement of RNA. It is appropriate within clinical trial, but is very difficult for routine practice. No correlations were observed between mutations in the TERT promoter and BCa features. In kidney cancers, hTERT measured in tissue showed high accuracy for RCC while hTR in tissue and serum hTERT were less relevant. TERT promotor mutations showed important predictive value concerning survival and progression of ccRCC.

Last, in CaP both hTERT and hTR in tissue and serum showed high accuracy in cancer detection while the reports on prostatic secretions were of limited relevance.

Limitations. As in all systematic reviews, our study had to face the high variability of methods and objectives in the telomere literature. Having said that, the articles here reviewed reported on high quality prospective trials that all pointed toward the clinical relevance of telomere biology in genitourinary malignancies. Unfortunately, few studies report absolute levels of the biomarkers and locus of mutation, while the majority compare the results between cancer and control groups, reporting relative difference. This fact, as well as different methodology, makes it impossible to compare different cancer types.

5. Conclusions

hTERT showed high accuracy in genitourinary cancers, while the value of hTR was more controversial. hTERT and TERT promotor mutations may have predictive value for BCa and RCC staging and grading, while no such relationship was observed in CaP. Although telomerase subunits showed clinically relevant values in genitourinary cancers, developing fast and cost-effective methods is required before contemplating routine use.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2021.01.022.

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