Emerging targets in upper tract urothelial carcinomas: the TERT gene

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Introduction: *Urothelial carcinoma (UC) is the fourth most prevalent malignancy in adults, accounting for 2.1% of cancer-related deaths. We aimed to describe the most frequent telomerase reverse transcriptase (TERT) gene mutations in this type of cancer and their relationship with the prognosis and treatment of this disease.*

Materials and methods: *We performed a search strategy in Medline and Embase with the following keywords: telomerase reverse transcriptase (TERT) gene and upper tract UC. We included reviews and observational studies to support the statements throughout the manuscript.*

Results: *The transcriptional activation of the TERT gene and subsequent telomerase activity is a prerequisite step in malignant transformation and progression. In advanced upper tract UC, TERT mutations are the most common genomic alterations in the Foundation Medicine database. C228T mutations predict distant metastasis in 60% of patients with renal pelvis cancer and 11% with ureteral cancer. Also, C228T and C250T mutations in urine DNA had a sensitivity of 87% and specificity of 94.7%. All TERT genomic alterations are inactivating short variant sequence mutations. There are no copy number gains or losses in TERT and no TERT gene rearrangements or fusions.*

Conclusions: *Multiple markers, and mutations regarding the TERT gene and its promoter have been found in upper tract UC. The C250T and C228T mutations have shown promising results as diagnostic markers detected with urine tests.*

Key Words: urothelial carcinoma, upper tract, TERT gene, mutation

Introduction

Urothelial carcinoma (UC) is the most common tumor of the urinary tract.[1] It may originate in the lower urinary system (bladder and urethra) or the upper tract (ureter or pyelocaliceal system).[2] Bladder tumors comprise 90%-95% of UCs, thus being the most common urinary malignancy.[3,4] In contrast, upper tract UCs are uncommon and account only for 5%-10% of cases of urothelial cancer; two per 100000 inhabitants in western countries.[5]

Upper tract UC typically occurs in more advanced stages when compared with bladder UC. It is more common in people between 70-90 years, and it is three

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to four times more common in men.[6] Furthermore, the survival rate is lower in women than in men.[7]

The histology involved in these primary tumors might present as urothelial, adenocarcinomas, and squamous cell carcinomas in decreasing order of incidence. Squamous cell carcinomas account for approximately 8% of tumors of the renal pelvis.[8] These tumors are associated with a lower survival than UCs because they tend to present in more invasive stages. They are also associated with urolithiasis and/or chronic infection.

There are different modifiable risk factors associated with upper tract UC, such as living in Balkan countries (Bulgaria, Greece, Romania, Yugoslavia); dietary exposure to aristolochic acid was reported, which is a potent carcinogen derived from plants of the genus Aristolochia;[9] and cigarette smoking (relative risk of 2.5 to 7).[10] On the other side, non-modifiable factors, such as hereditary genetic risk factors, show a close association with lynch disease.[11]

When suspecting upper tract UC, invasive procedures such as retrograde pyelography, computed tomography (CT) urography, or flexible ureteroscopy with biopsy are performed to confirm diagnosis. Currently, urine cytology is the only non-invasive diagnostic test for upper tract UC approved by practice guidelines. Novel approaches including ctDNA detection in catheterized urine samples are in various stages of development.[12,13] Although the sensitivity of urine cytology for the detection of upper tract UC is as low as 40% in some studies, for others it has a much higher yield.[14]

It is now recognized that the *TERT* gene plays an essential role in the natural history, and the progression of upper tract UC.[15,16] Consequently, it is essential to find more specific biomarkers to identify the population at risk, to provide a timely and early diagnosis, to improve its prognosis, and to offer personalized treatment.

The expression of *TERT* and its association with UC

TERT is a critical catalytic component for cellular homeostasis. It is responsible for lengthening telomeric DNA.[16] The *TERT* gene is transcriptionally repressed and telomerase is silent in most normal human somatic cells, while *TERT* induction along with telomerase activation is necessary for malignant transformation and occurs widely in human cancer, including upper tract urothelial cancer.[17,18] It is well established that aberrant expression of *TERT* confers not only unlimited proliferative potential by stabilizing telomere size, but also aggressive phenotypes through their independent telomere lengthening in cancer cells.[19-22] Given the fundamental role of *TERT* in oncogenesis, there has been focus on the association between single-nucleotide variants or SNPs of the *TERT* gene and cancer susceptibility. Rs2736100 (located in intron 2) is the most frequently studied, considering the risk of multiple types of cancer, as documented in many published reports.[23-27] Yuan et al revealed that rs2736100 AC predicted a reduced upper tract UC risk in a Chinese population.[28] However, it is currently unclear whether there is any link between this SNP and upper tract urothelial cancer.

The C228T and C250T *TERT* promoter mutations generate CCGGAA/T or GGAA/T, and de novo ETS binding motifs, promoting *TERT* transcription and telomerase activation.[29,30] Recently, the *TERT* promoter mutations (c228 (g.1295228 C>T in GRCh37), and c250T (g.1295250 C>T in GRCh37)) have been associated with multiple tumors, including urological malignancies.[14] Currently, members of the GABP subfamily act like a master key to facilitate the transcription of TERT and the activation of telomerase in cancer cells, Figure 1.[30] *TERT* mutation is present in 50% of patients with upper tract UC and 66% of bladder urothelial cancer.[14,31-34] The *TERT* promoter mutation frequently occurs in 99% precancerous lesions and low-grade urothelial bladder cancers,[32] high-grade urothelial bladder cancer, adenocarcinoma of the urinary bladder, squamous cell carcinoma of the urinary bladder, plasmacytoid urothelial carcinoma, and micropapillary urothelial carcinoma.[14]

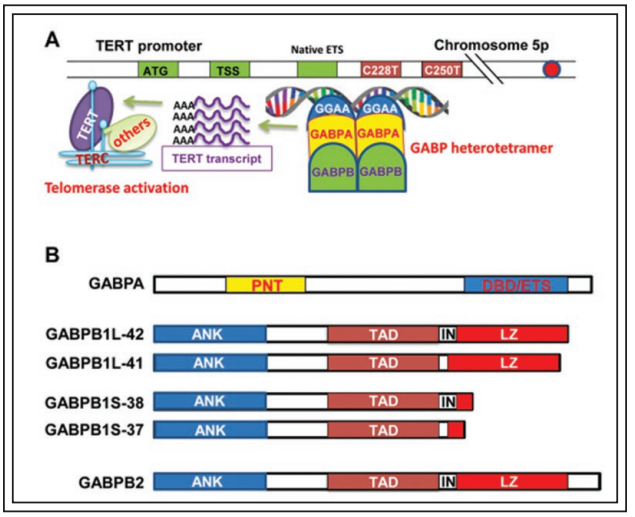

Figure 1. *TERT* promoter mutation.

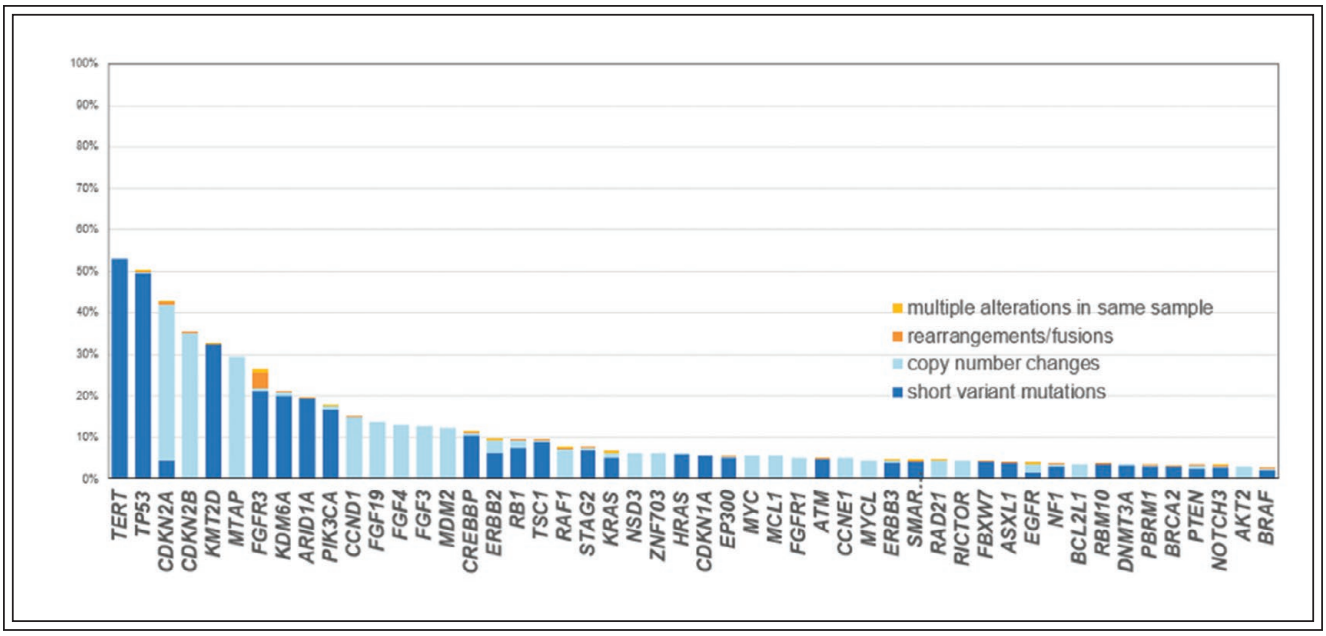

Figure 2a. Long tail plot of genomic alterations in upper tract urothelial carcinomas detected by hybrid capture-based comprehensive genomic profiling.

Consequently, the *TERT* promoter mutation has been established as a common genetic alteration in urothelial cancer. Kinde et al, found *TERT* promoter mutations in 66% muscle-invasive and 74% of non-muscle invasive bladder cancer.[14] This finding also supports the fact that *TERT* promoter mutation occurs as an early genetic event in urothelial carcinogenesis.

Upper tract UC

The upper tract UC include renal pelvis and ureteral carcinomas. The incidence is lower than urothelial bladder cancer but has increased over the past two decades. Upper tract UC is twice as often located in the renal pelvis than in the ureter. In about 20% of

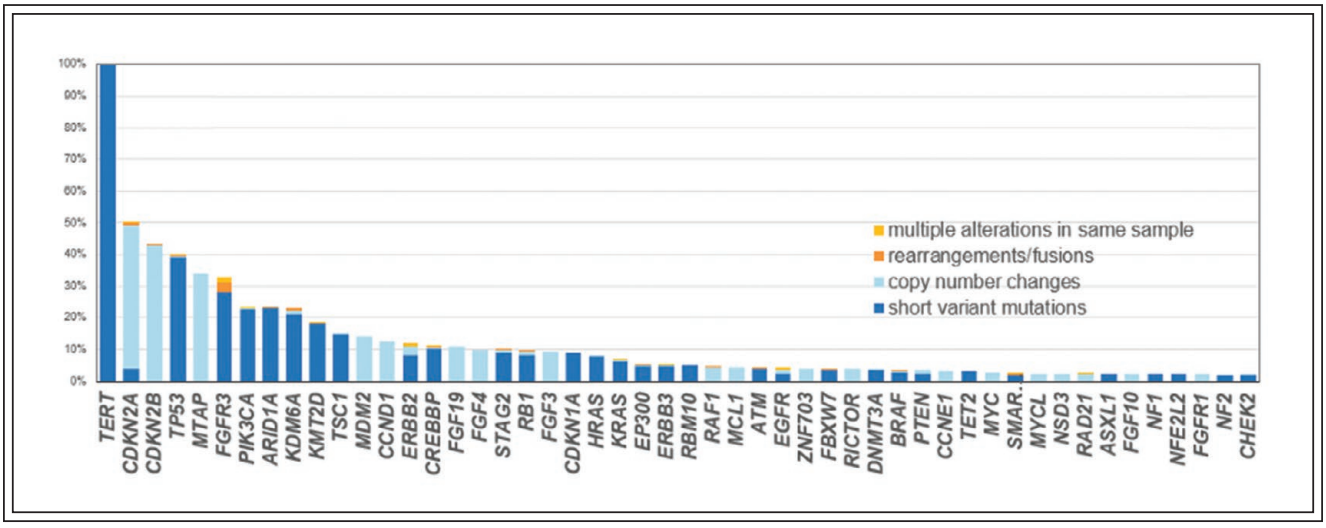

Figure 2b. Long tail plot of genomic alterations in *TERT* mutated upper tract urothelial carcinomas detected by hybrid capture-based comprehensive genomic profiling.

cases, concomitant bladder cancer is present.[35] Upper UCs are invasive in 60% or metastatic in approximately 25% at the time of diagnosis,[36] mainly due to the lack of early clinical symptoms and specific non-invasive diagnostic testing modalities.

The transcriptional activation of the *TERT* gene and subsequent telomerase activity is a prerequisite step in malignant transformation and progression.[37] Currently, this gene has gained ground with its presence in multiple human malignancies, including upper tract UCs and bladder carcinoma.

For patients with clinically advanced and stage IV upper tract UC, *TERT* mutations are the most common genomic alterations in the Foundation Medicine database of 1,401 cases. All *TERT* genomic alterations are inactivating short variant sequence mutations. There are no copy number gains or losses in *TERT* and no *TERT* gene rearrangements or fusions. *TERT* genomic alterations are identified in 53% of the cases. All *TERT* alterations are short variant mutations. *CDKN2A* is inactivated by homozygous deletion or inactivating mutation in 42.8% of cases with *MTAP* homozygous deletion in 29.4% of cases. *FGFR3* genomic alterations are identified in 26.6% of cases, Figure 2a. In addition, all (100%) of the *TERT* genomic alterations are short variant mutations. When compared with all upper tract cases, Figure 2a, the *CDKN2A* genomic alteration frequency has increased to 50.6% of cases, *MTAP* homozygous deletions have increased to 34.1% and *FGFR3* genomic alterations have increased to 32.9% of cases, Figure 2b.

In *TERT* mutated upper tract UC, noteworthy alterations in other genes important for urothelial cancer management include increased *CDKN2A* loss which may impact immune checkpoint inhibitor response and slightly increased alterations in *FGFR3* associated with approved targeted therapy options.

Necchi et al found that there were multiple genomic differences among bladder UC and upper tract UC. *TERT* promoter was the most frequently mutated gene in both cancers; however, it was most frequent in bladder UC (68%) than upper tract UC (47%, $p < 0.001$). Moreover, there were other genomic alterations regarding *FGFR3* short variant (30% vs. 13%) and *HRAS* short variant (7.3% vs. 3%) that might differentiate between upper tract and bladder UC. Furthermore, *HRAS* short variant may be enriched in renal pelvis (9.5%) and ureteral (1.8%, $p = 0.002$) UC. Another important issue is that upper tract UC was enriched for microsatellite instability (MSI) high (3.4%) relative to bladder UC (0.8%, $p < 0.001$).[38] Although these are not specifically genomic alterations described in this review, they are important to support the peculiarities among both types of UC, and between renal pelvis and ureteral UC. A routine incorporation of ctDNA assays in clinical trials is essential when looking beyond regarding the best diagnosis and treatment of these tumors.

Diagnosis and prognosis

The presence of *TERT* promoter mutations is closely associated with aggressive or invasive. In the upper tract UC was identified C228T mutations in 60% with renal pelvis cancer and 11% with ureteral cancer, predicting distant metastasis.[39] Furthermore, several researchers reported that *TERT* promoter mutation is associated with bladder recurrence and worse survival in urothelial bladder cancer, not only in the upper tract.[40,41] Isharwal et al reported that the *TERT* promoter mutation in bladder UC was associated with worse overall survival (HR: 2.31, 95% CI: 1.46-3.65), disease-specific survival (HR: 2.23, 95% CI: 1.41-3.53), and metastasis-free survival (HR 1.63, 95% CI: 1.05-2.53); also, worse results for upper tract UC: overall survival (HR: 11.8, 95% CI: 4.68-30.02), disease-specific survival (HR: 16.03, 95% CI: 5.64-45.54), and metastasis-free survival (HR 8.93, 95% CI: 4.74-26.66). However, authors also stated that tumors with a higher mutational burden per megabase had a more favorable outcome.[41]

Urine cytology is a non-invasive diagnostic method that is useful for the diagnosis of high-grade bladder cancer. However, it has little or no utility in low-grade bladder cancer, this added to the other costly and invasive diagnostic methods, such as endoscopies and biopsies, has been looking for diagnostic methods with better values of sensitivity and specificity with blood and urine samples in the genetic identification of these mutations to make an earlier diagnosis.[32,42] Moreover, the *TERT* mutated could be detected in urine from those patients with the mutation-positive, which can serve as a urine-based diagnostic marker.[39]

The usefulness and clinical application of urine pellet analysis using a panel of specific sequences "UROSEEK" which includes a mutation in the *TERT* gene has been demonstrated, showing good results with a sensitivity of 68% (95% CI, 59% to 73%) and the combination of UROSEEK plus cytology reached 71% sensitivity (95% CI, 61.48% to 78.77) in high-grade and low-grade urothelial cancer, offering promising data with this type of diagnosis.[43] Researchers have agreed with these data on *TERT* gene mutation and its presence in the bladder and high-grade urothelial cancer (92%, cfDNA in UBC; 89%-94%, urine pellet in UTUC; 73%-90%, urine pellet in UBC).[44]

Multiple studies have been found that show the efficacy of next-generation DNA sequencing in urine

in urothelial cancer patients. There was an association between the *TERT* promoter and *FGFR3* with bladder cancer (sensitivity of 68.9% and a specificity of 100%). Furthermore, the presence of the *TERT* gene is greater than 14% in patients with a worse prognosis and recurrence of bladder cancer in patients after transurethral surgery for non-muscle invasive bladder cancer or radical nephrectomy for high urothelial cancer.[45]

Avogbe et al revealed that the presence of C228T and C250T mutations in urine DNA had a sensitivity of 87% and specificity of 94.7%. There was evidence of improved diagnosis when combined with urinary cytology and good results in diagnosing early stages of urothelial cancer.[46] It suggests the use of these DNA samples in urine and future research in this field.

Other mutations of the *TERT* gene were found in the *PLEKHS 1* promoter and GPR126 intron six. However, it was found that their presence is associated more with bladder cancer and less frequently with high urothelial cancer. Nonetheless, these markers are found in urine, which is not generally produced by urothelial tissue.[47]

Concerning the quantification or localization of these mutations in plasma is low in non-muscle invasive tumors, it has been shown that with this technique in blood samples patients are not detected in early stages, only have shown the presence of these in metastatic stages of urothelial cancer, the mutation was found in high metastatic urothelial cancer 17% and in bladder cancer 10%-23%.[46,48]

Furthermore, Christensen et al[49] showed a significant association between ctDNA level and tumor grade. Consequently, the amount of ctDNA from bladder tumor patients who were mutation-positive was significantly associated with progression-free survival or recurrence-free survival.[47]

TERT as a potential therapeutic target

IN-5401 and INO-9012 are mixtures of synthetic plasmids, which include the *TERT* antigen. These were administered in a phase I/II study to patients with metastatic UC combined with atezolizumab (NCT03502785). However, this combination had limited success. According to the literature, *TERT* promoter mutations occur early in tumorigenesis; therefore, these therapies may be effective for prevention of UC recurrence or early-stage UC.[50]

Conclusion

In conclusion, multiple markers, and mutations regarding the *TERT* gene and its promoter have been found in upper tract UC. The C250T and C228T mutations have shown promising results as diagnostic markers detected with urine tests, and their combination with cytology demonstrate promising sensitivity. Diagnostic techniques should be optimized to provide the population with timely and appropriate diagnosis and treatment. □

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