Comparison of Genomic Characterization in Upper Tract Urothelial Carcinoma and Urothelial Carcinoma of the Bladder

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Abstract

Background: Different genomic characterization in urothelial carcinoma (UC) by site of origin, may imply contrasting therapeutic opportunities and pathogenetic mechanisms. The aim of this study was to investigate whether the differences between upper tract urothelial carcinoma (UTUC) and urothelial carcinoma of the bladder (UCB) result from intrinsic biological diversity.

Methods: We prospectively sequenced 118 tumors and matched blood DNA from Chinese UC patients using next-generation sequencing (NGS) techniques, including 45 UTUC and 73 UCB.

Results: There were marked disparities in the mutational landscape for UC according to race and site of origin. Signature 22 for exposure to aristolochic acid (AA) and signature 10 for defects in polymerase *POLE* were only observed in the UTUC cohort. Conversely, signature 6 for defective DNA mismatch repair only existed in the UCB cohort. Compared to UCB, UTUC had higher clonal (p<0.001) and subclonal mutation numbers (p=0.015). *TP53*, *PIK3CA*, and *FGFR3* mutations may be the driver genes for UTUC, whereas for UCB, the driver gene may be *BRCA1*. UTUC patients had lower PD-L1 than UCB patients. There was no significant difference in the number of DDR mutations, copy number variation (CNV) counts, tumor mutational burden (TMB) or clinical actionability between UTUC and UCB.

Conclusions: UTUC and UCB exhibit significant differences in the prevalence of common genomic landscape and carcinogenesis. Consequently, molecular subtypes differ according to location, and these results may have important implications for the site-specific management of patients with urothelial carcinoma. Mutational signature may be used as a screening tool to assist clinical differential diagnosis between UTUC and UCB.

Introduction

Urothelial carcinoma (UC) is the twelfth most common malignancy worldwide, originating from the bladder and upper urinary tract, including the renal pelvis and ureter (1). There is similar histologic appearance between upper tract urothelial carcinoma (UTUC) and urothelial carcinoma of the bladder (UCB), however, they have distinct epidemiologic and clinicopathologic differences (2). UCB accounts for 90–95% of all UCs, whereas UTUC accounts for only 5–10% (3).

Compared to UCB, because of the relative rarity of UTUC, clinical decision-making for patients with UTUC is extrapolated based on treatment data for UCB, which is focused predominantly or exclusively on by most clinical and biologic studies (4). However, UTUC tends to have a worse prognosis with 5-year cancer-related survival of < 50% for pT2/pT3 tumors and < 10% for pT4 tumors. For UCB, the 5-year overall survival was 66% and 5-year recurrence-free survival was 76% in patients with pT1 tumors, 74% for pT2, 52% for pT3, and 36% for pT4 (5). Molecular approaches contribute to enhanced understanding of cancer biology. Genomic profiles of UCB have been examined by multiple recent studies, which improves knowledge about diagnosis, prognosis and therapeutic targets, whereas UTUC is less well characterized, especially UC in China (6). Small retrospective case series confirmed diversity in the

prevalence of gene mutations between UCB and UTUC (7). It is crucial for understanding the similarities and differences in the genetic characteristic of UTUC and UCB to define treatment strategies.

Based on these considerations, the aim of this study is to evaluate the molecular differences and to explore the potential clinical utility of tumor genomic characterization to guide clinical management between UTUC and UCB in China through next-generation sequencing (NGS). We were particularly interested in determining whether there were differences in molecular mechanisms and opportunities for immunotherapy and targeted therapy between UTUC and UCB.

Methods

Patient enrollment and study design

One hundred eighteen patients with UC were recruited at The First Hospital of Peking University hospital between January 2014 and April 2019, including 45 UTUC and 73 UCB patients. Stages ranged from I to IV, which was confirmed according to AJCC staging (8th edition). Informed consent was obtained from all participants. This study was approved by the Ethical Committee of The First Hospital of Peking University. A total of 319 UTUC and 350 UCB patients for Western TCGA were acquired from the cbioportal. (http://www.cbioportal.org/).

Library preparation and Next-Generation Sequencing

Tissue DNA and blood controls were extracted using the QIAamp Genomic DNA kit (Germany, QIAGEN) according to the manufacturer's instructions. Sequencing libraries were constructed by Illumina standard library construction instructions (Illumina, Inc.). NGS was performed using the 808 cancer-related gene panel, which is certified for clinical use, and patient reporting designed by Acornmed. The target-enriched libraries were sequenced according to Illumina HiSeq2500 NGS platform (Illumina, Inc.). The sequencing depth was >10,000×. Sequence reads were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler alignment (BWA) tool (8). Local realignment and base quality score recalibration was performed by GATK software (version 2.3; software.broadinstitute.org/gatk) (9). MuTect2 (version 1.1.1; software.broadinstitute.org/cancer/ cga/mutect) with the recommended parameters was used to identify single-nucleotide variants (SNVs) and small insertions or deletions (INDELs) (10). CONTRA software (version 2.0.4; contra-cnv. sourceforge. net) was performed to analyze copy number variant (CNV) calling, including homozygous deletions and amplifications (11). The cutoff value for high- and low-CNV of our cohorts was defined as the median CNV.

Tumor Mutational Burden Definition

TMB was defined as the number of somatic, coding, indel mutations and base substitutions per megabase (Mb) of genome examined, including synonymous mutations, nonsynonymous mutations, and frameshift mutations. TMB score was calculated by the total number of mutations counted/2 Mb. Mutations with allelic fractions of less than 0.01 or coverage of $\leq 50\times$ were excluded. The cutoff value

for high- and low-TMB of our cohorts was defined as the median TMB, which was referred to in the previous article (12).

DNA damage repair (*DDR*) gene mutation analysis

The 34 *DDR* gene status was analyzed, which was previously identified as related with response to PD-1/PD-L1 blockade (13). Those genes participated in several *DDR* canonical pathways, including nucleotide excision repair (NER), Mismatch repair (MMR), Fanconi Anemia (FA), homologous recombination (HR), checkpoint and others (Table S1).

Enrichment Analysis

KOBAS-Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was used by "clusterProfiler, org.Hs.eg.db, plot, ggplot2" in R package (14).

Mutational signature analysis

Mutational signature analysis was identified using nonnegative matrix factorization (NMF) from the 96-channel mutational profiles following the authors' guidelines (15). Discovered mutational signature was compared with 30 COSMIC signatures using hierarchal clustering of cosine similarity among those signatures with 'ward. D2' linkage.

Inferring the clone or subclone muation by Cancer Cell Fraction (CCF)

Clonal mutation was detected using ABSOLUTE, according to the value of the CCF, which was the fraction of tumor cells carrying this mutation within a sequencing sample (16). Mutation was classified as clonal if the estimated CCF was >0.9 and the *Pr* (*clonal*) was >0.5, and as subclonal otherwise (17).

Identification of oncodrive genes

Oncodrive genes are defined by providing selective growth advantage to cancer cells when genetically altered. Oncodrive genes were determined by OncodriveCLUST algorithm, which leverages the observation that a majority of the activating mutations within oncogenes are clustered around mutational hotspots (18).

Statistical analysis

SPSS 22.0 software (New York, IBM Corporation) was used to perform the statistical analysis. Fisher's exact test, one-way analysis of variance and Kruskal-Wallis test were used to determine the differences in the clinical data in mutations between the UTUC and UCB cohorts. Probability values were derived from two-sided tests and values of $p < 0.05$ were considered significant.

Results

Patient demographic and clinical data

One hundred eighteen patients with UC were enrolled, including 45 patients with UTUC and 73 patients with UCB. Patient characteristics are summarized in Table 1. The UCB patient cohort had a higher ratio of males to females and PD-L1 expression than the UTUC patient cohort (5.08:1 vs. 1.81:1, p = 0.025 and 31.51 vs. 17.78, p = 0.003, respectively). A total of 8.89% (4/45) of patients with UTUC and only 2.74% (2/73) of patients with UCB (p = 0.661) had dMMR. There was no significant differences in other characteristics in both cohorts.

Table 1
Characteristics of patients with urothelial carcinoma

Characteristics	UTUC (n, %)	UCB (n, %)	P value
N	45	73	
Sex			0.025
Male	29 (64.44)	61 (83.56)	
female	16 (35.56)	12 (16.44)	
Age			0.342
> 65 year	22(48.89)	43 (58.90)	
≤ 65	23 (51.11)	30 (41.10)	
Smoking			0.231
Yes	13 (28.89)	30 (41.10)	
No	30 (66.67)	39 (53.42)	
Missing	2 (4.44)	4 (5.48)	
ECOG			0.621
0	1 (2.22)	1 (1.37)	
1	20 (44.44)	38 (52.50)	
2	4 (8.89)	4 (5.48)	
Missing	20 (44.44)	30 (41.10)	
Stage			0.225
I	5 (11.11)	6 (8.22)	
II	11(24.44)	14 (19.18)	
III	14 (31.11)	21 (28.77)	
IV	7(15.56)	18 (24.66)	
Missing	8 (17.78)	14 (19.18)	
Metastasis			0.531
Yes	11(24.44)	23 (31.51)	
No	31 (68.89)	48 (65.75)	
Msissing	3 (6.67)	2 (2.74)	
Treat-naive			0.671

Characteristics	UTUC (n, %)	UCB (n, %)	P value
Yes	31 (68.89)	48 (65.75)	
No	11(24.44)	22 (30.14)	
Msissing	3 (6.67)	3 (4.11)	
Recrudesce			0.832
Yes	11(24.44)	24 (32.88)	
No	31 (68.89)	46 (63.01)	
Msissing	3 (6.67)	3 (4.11)	
PD-L1 expression			**0.033***
≤ 1%	22 (48.89)	21 (28.77)	
> 1%	8 (17.78)	23 (31.51)	
Msissing	15 (33.33)	29 (39.73)	
MMR			0.661
dMMR	4 (8.89)	2 (2.74)	
pMMR	16 (35.56)	17 (23.29)	
Missing	25 (55.56)	54 (73.97)	

Landscape of mutations in UTUC and UCB

To explore the somatic mutation patterns in both UTUC and UCB, we conducted Acornmed 808 panel to identify somatic mutations. One thousand six hundred fifty nonsynonymous protein coding mutations were found in 45 patients with UTUC, ranging from 4 to 333 mutations. Patients with UCB harbored 2017 nonsynonymous protein coding mutations in total, ranging from 11 to 255 mutations. The top 20 most frequently mutated genes were identified in UTUC and UCB. *KMT2D*, *TP53*, and *BRD4* mutations were the most frequent in both UTUC (Figure 1A) and UCB (Figure 1B). Eight genes were significantly different in diverse tumor location (Figure 1C). Notably, *INRRL1* mutations only occurred in UCB. However, compared to UCB, mutations of *KMT2C*, *LRP1B*, *NCOR1*, *ZFHX4*, *KDR*, *NF1*, and *NOTCH* genes were more likely to be seen in UTUC. In addition, we discovered that 20 genes were significantly different between our *UTUC cohorts* and TCGA UTUC cohorts, such as *KMT2D*, *TP53*, and *FGFR3*. Moreover, 11 frequently mutated genes were significantly different between our UCB cohorts and TCGA UCB cohorts, such as *KMT2C*, *FAT1*, and *GANQ*.

Prevalence of mutated genes is related to clinical stage

We attempted to determine whether the prevalence of mutated genes was correlated with clinical stage in UTUC and UCB. The significantly different genes in UTUC and UCB, as well as the top 5 mutated genes,

were analyzed for various stages, which were divided into two groups by stage (clinical stage I and II vs. stage III and IV), referring to the previous article ([7]). There was no difference in the genes that were identified as significantly different between UTUC and UCB in various stages (Figure S1A). Surprisingly, there were significant differences for the prevalence of the top 5 genes in various stages and cancer types (Figure S1B-E). For stage Ⅰ and Ⅱ, *SPEN* mutation was more frequent in UTUC than that in UCB (38% vs. 0%, p=0.004). Nevertheless, *FGFR3* mutation was also more frequent in UCB (19% vs. 53%, p=0.004), as previous studies showed. For stage Ⅲ and Ⅳ, *ARID1A* mutation was more frequent in UTUC than that in UCB (32% vs. 11%, p=0.039). Interestingly, there was no significant difference for gene mutations at various stages in UTUC, although the frequency of *SPEN* mutation seemed to be higher in stage Ⅰ and Ⅱ than that in stage Ⅲ and Ⅳ (38% vs. 11%, p=0.055). For UCB, *FGFR3* mutation was significantly more frequent in stage Ⅲ and Ⅳ than that in stage Ⅰ and Ⅱ (53% vs. 5%, p<0.001). Conversely, *SPEN* and *PTPRT* mutations were significantly more frequent in stage Ⅲ and Ⅳ (0% vs. 21%, p=0.042 and 0% vs. 24%, p=0.022, respectively).

Mutational signature for UTUC and UCB

To investigate the molecular characteristics of UTUC and UCB, mutational signature and somatic substitution analysis were undertaken. C to T (C>T) substitutions were the dominant mutation type in both UTUC and UCB (36.5% and 44.0%, respectively). For UTUC, the transversions, which are interchanges of purine for pyrimidine bases involving the exchange of one-ring and two-ring structures, were dominant DNA substitution mutations (Figure 2A). Whereas transitions that are interchanges of two-ring purines (AG) or of one-ring pyrimidines (CT), involving bases of similar shape, were dominant DNA substitution mutations in UCB (Figure 2B). In addition, only signature 1 for spontaneous deamination of 5−methylcytosine existed in both cohorts (41.86% and 26.61%, respectively). Although there were *APOBEC* Cytidine Deaminase signatures in both groups, they were projected to different COSMIC signatures (signature 2 and 13, respectively), with a difference rate (32.41% and 42.99%, respectively). Signature 22 for Exposure to aristolochic acid (AA) (20.91%) and signature 10 for defects in polymerase *POLE* (4.81%) were only observed in the UTUC cohort (Figure 2C, E). Conversely, Signature 6 for defective DNA mismatch repair (30.41%) only existed in the UCB cohorts (Figure 2D, F). In addition, 5−methylcytosine mutational signature was the predominant mutational signature seen in UTUC cohorts, but *APOBEC* was the mutation signature for UCB cohorts.

KEGG enrichment for UTUC and UCB

To better understand the differences in biological functions between UTUC and UCB, KEGG enrichment analysis was performed. In the UTUC cohort, mutated genes were mainly enriched in the cancer pathway, PI3K-Akt signaling pathway, ErbB signaling pathway, HTLV-I infection and Rap1 signaling pathway (Figure 3A). Regarding the UCB cohort, the primary pathways included the cancer pathway, PI3K-Akt signaling pathway, HTLV-I infection, FoxO signaling pathway, and Thyroid hormone signaling pathway (Figure 3B). Rap1 signaling pathway was not discovered in UCB cohorts. To study the proportion of mutational pathways in the UTUC and UCB cohorts further, we analyzed 10 oncogenic signaling

pathways reported in previous literature (19). There were no significant differences for the frequency of oncogenic signaling pathways between UTUC and UCB (Figure 3C). The common pathways were the RTK/RAS pathway (68.89% vs. 75.34%), Notch pathway (62.22% vs. 68.90%), p53 pathway (60.00% vs. 58.90%), PI3K pathway (51.11% vs. 52.05%), and Hippo pathway (35.56% vs 38.36%).

Co-occurrence and mutual exclusion among genetic events

To identify correlations among different genes in two UC subtypes, co-occurrence and mutual exclusion were performed for the top 10 gene in each cohort. We discovered that only *FGFR3* mutations were mutually exclusive with *TP53* mutations in both UTUC and UCB. In the UTUC cohorts, *CREBBP* mutations were significantly associated (co-occurred) with *FAT1*, *FGFR3*, and *KMT2C* mutations (Figure S2A). Similarly, *KMT2C* mutations co-occurred with *SPEN* and *KMT2D* mutations. In addition, *NCOR1* mutations co-occurred with *LRP1B* mutations. Regarding the UCB cohorts, *TP53* mutations were associated with RB1 mutation and were mutually exclusive with *KMT2A* and *ERCC2* mutations. *KMT2C* mutations co-occurred with *FAT1* and *KMT2A* mutations. Analogously, there were co-occurrences for *ERCC2* with *FAT1*, and *GNAQ* with *BRD4* mutations (Figure S2B).

Inference of mutation clonality for UTUC and UCB

To investigate differences of clonal evolution in UTUC and UCB, we thus inferred the clonal status. Combined with the analysis of all somatic cell mutations, including nonsynonymous SNVs and splice site (generally referred to as nonsilent) and synonymous SNVs (referred to as silent) within exonic, splicing, intergenic and intronic regions, we found that there were 2,040 clonal mutations and 22,330 subclonal mutations in UTUC. Regarding UCB, there were 195 clonal mutations and 2,482 subclonal mutations. Subsequently, clonal status was evaluated by nonsilent mutation, which may play a role in carcinogenesis. Five hundred twenty-seven clonal mutations and 287 subclonal mutations were identified in 82.22% (37/45) of UTUC patients. There were 34 clonal mutations and 106 subclonal mutations in 56.16% (41/73) of UCB patients. To our surprise, UTUC had higher clonal (p<0.001) and subclonal mutation numbers (p=0.015) than those in UCB (Figure 4A). There was a significant difference for the prevalence of genes of clonal mutations between UTUC and UCB. For example, clonal and subclonal mutations of *KMT2D*, *KMT2C*, *PIK3CA* and *FAT1* existed only in UTUC (Figure S3).

Clonal driver mutations of Genomic Events

Cancer drivers only occur in a minority of somatic mutations, which confer clonal fitness and are positively selected over the course of tumor evolution (20). Clonal mutations represent the early events in tumor evolution. Conversely, subclonal mutations represent relatively late events based on occurring after the emergence of the most recent common ancestor (21). Thus, clonal driver mutation might contribute to identifying potential therapeutic strategies (20). In this study, we explored the clonal driver gene by the nonsilent with clonal mutation. Interestingly, driver genes were significantly different between UTUC and UCB. *TP53*, *PIK3CA*, and *FGFR3* mutations are the driver genes for UTUC (Figure 4B), whereas for UCB, the driver gene only was *BRCA1* (Figure 4C).

Assessment of DDR gene mutation suggesting potential benefit form immunotherapy

Thirty-four *DDR* genes related to the response of PD-1/PD-L1 blockade and sensitivity to cisplatin-based regimens were analyzed (13). Twenty-six deleterious *DDR* gene alterations were observed in 60% (27/45) of UTUC patients, with a median of 2 *DDR* alterations per patient (range, 1 to 10). There were 27 *DDR* gene alterations in 71.23% (52/73) of UCB patients, with a median of 2 *DDR* alterations per patient (range, 1 to 14). There was no difference for the frequency of *DDR* mutations between UTUC and UCB (60% vs. 71.23%, p=0.572). In UTUC, *BRCA2* (n=9), *ERCC2*, and *ATM* (n=7 each) were the most frequently mutated genes, while in UCB, the most frequently mutated genes were *ERCC2* (n=13), *BRCA2*, and *MDC1* (n=12 each) (Figure 5A). The most commonly interfered pathways or mechanisms related were FA (40% and 47.95%), Checkpoint (37.78% and 43.84%) and MMR (31.11% and 31.51%) pathway in both UTUC and UCB. *ERCC4* mutations were more frequent in UTUC than those in UCB (p=0.019). There were not significant differences in both frequency and number of *DDR* mutations (p=0.231) and other mutated genes (p=0.208) in both cohorts.

Pattern of somatic CNV in UTUC and UCB

It has been demonstrated that CNV can be used to categorize tumors into distinct sensitivity to ICI therapy (22). In this study, *ROS1*, *EGFR*, *BRCA1*, and *NTRK1* mutations were the most commonly found in UTUC (Figure S4A) and UCB (Figure S4B), with *ROS1* mutations appearing to be more frequent in UTUC than UCB (p=0.055). Conversely, patients with UTUC had less LOC284294 mutations than UCB patients (Figure 5B). The median CNV counts were 49.62 (range from 0 to 706.54) for UTUC and 48.00 (range from 0 to 537.89) for UCB. There were no significant differences for CNV numbers in either cohort (p=0.511, Figure 5C).

TMB comparison between UTUC and UCB

To determine whether the TMB value in UTUC and UCB can be used to screen the potential beneficiaries for immunotherapy, we compared differences in TMB between the two groups. The median TMB was 13.31 (range from 0.89 to 117.46) for UTUC and 15.76 (range from 3.99 to 62.04) for UCB. There were no significant differences in TMB in either cohort (p=0.489, Figure 5D).

Relationship among immune markers for DDR mutation, CNV, TMB, and PD-L1

We further explored the correlation between immune markers, including TMB, PD-L1 expression, and CNV and *DDR* gene mutation. In this study, we discovered that *DDR* gene alterations were associated with higher CNV counts in the UTUC cohort (p<0.001, Figure S5A). However, this correlation was not found in the UCB cohort (p=0.435, Figure S5A). Surprisingly, *DDR* gene alterations were associated with higher TMB in both UTUC and UCB tumors (p<0.001, p<0.001, respectively, Figure S5B). Interestingly, UTUC patients with lower CNV counts had a higher TMB than those with higher CNV counts (p=0.009, Figure S5C). By contrast, higher TMB was more common in UCB patients with high CNV (p=0.006, Figure S5C). Patients undergoing PD-L1 detection were selected to analyze the relationship. We discovered that PD-L1

was not associated with *DDR* gene mutation, CNV counts or TMB in both UTUC and UCB cohorts (p>0.05, Figure S3D-F).

Assessment of clinical actionability suggesting potential benefit from target therapy

To explore whether there are similar principles in the treatment of UTUC based on UCB and clinical utility for prospective molecular profiling to guide treatment, clinical actionability was evaluated using OncoKB (http://oncok b.org/). We found that 37 (82.22%) UTUC patients harbored at least one actionable alteration, covering 53.33% (24/45) of patients with the targeted drug (Figure 6A). Sixty (82.19%) patients with UCB harbored at least one actionable alteration, including 54.79% (40/73) of patients with the targeted drug (Figure 6B). The rates of receiving targeted drugs were similar in both groups, whereas *KIT*, *NRAS*, and *CDKN2A* only existed in UTUC (Figure 6C), and *EGFR*, *MDM2*, *CDK4*, and *BRCA2* in UCB (Figure 6D).

Discussion

UTUC and UCB show epidemiologic and clinical differences; Notwithstanding, there exists a similar histologic appearance (23). By comparing the mutational profiles of UTUC and UCB, we discovered distinct patterns of mutations, mutational signatures, clones and subclonal mutation states as well as driver genes. Moreover, we found that they may have similar opportunities for immunotherapy and targeted therapy.

In this study, we systematically explored the landscape of mutations in UTUC and UCB and discovered that several genes had markedly different mutation frequencies. Compared to those in UCB, *KMT2C*, *LRP1B*, and *NCOR1* were significantly more frequent mutations in UTUC. Whereas *INPPL1* mutations only occurred in UCB. The significant differences in the mutational frequency of those genes remained after adjusting for clinical stage. This result might contribute to the design of targeted therapy trials for different pathologic subtypes of UC. In addition, there were significant differences in both UTUC and UCB between Asian and Western patients, which suggests that deciphering the differences for the molecular mechanism of UC among various others is conducive to the understanding of the mechanisms of carcinogenesis, the realization of precision therapy, and the expansion of the benefit to the population.

Mutational signature within cancer genomes reveals the diversity and complexity of somatic mutational processes underlying carcinogenesis (24). We found distinct mutational signatures between UTUC and UBC, which may suggest the different mechanisms of carcinogenesis. The *APOBEC* mutational signature was the predominant mutational signature seen in UCB cohorts (42.99%), but 5 – methylcytosine mutational signature was the most frequent for UTUC (41.86%). *APOBEC* mutational signature has been found in 22 cancer types, where activation of AID/APOBEC cytidine deaminases is due to viral infection, retrotransposon jumping or to tissue inflammation, which was consistent with other studies (25, 26). The COSMIC mutational signature database indicates that the 5 – methylcytosine mutational signature has been found in several cancer types and is the result of an endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine, which is associated with the aging of cancer diagnosis

(27–30). To our knowledge, we are the first to report that the 5-methylcytosine mutational signature was the predominant mutational signature seen in UTUC cohorts. Lu et al. demonstrated that the AA mutational signature was associated with AA exposure, which could be a screening tool defining low-risk UTUC with therapeutic relevance (31). POLE encodes subunits of Polε DNA polymerase enzyme complex, which serves the major catalytic and proofreading and replicates the leading strand during DNA replication. POLE mutational signature is related to recurrent *POLE* somatic mutations, which may suggest that DNA replication is associated with errors in proofreading activity of Pol ε (32). DNA mismatch repair mutational signatures are usually microsatellite-unstable, serving as a better indicator of response (33). In this study, AA and *POLE* mutational signatures were only observed in the UTUC cohorts. Conversely, defective DNA mismatch repair mutational signature was only seen in the UCB cohorts. Consistent with our conclusion, several reports have demonstrated that AA mutational signature was present for the upper urinary tract instead of the lower urinary tract (31, 34, 35). However, Poon et al. reported inconsistent results and have discovered the presence of AA mutational signature in bladder cancer in China, and it accounts for only 3% (3/99) of cases (36). One explanation for these finding is the difference in the enrolled population, because exposure to aristolochic acid is associated with other cancer types in addition to UC, such as cancers of the kidney, bile duct, and liver (37). Similarly, POLE mutational signature and defective DNA mismatch repair mutational signature are controversial (26, 31, 38, 39). These studies suggest that mutational characteristics may be influenced by a variety of factors and be used as a screening tool to assist clinical differential diagnosis between UTUC and UCB. In the future, more studies should be conducted to explore the mutational signature between UTUC and UCB, in order to better understand their carcinogenic mechanisms and to explore their role in stratification.

In this study, we discovered that UTUC had higher clonal and subclonal mutation numbers than those in UCB. To the best of our knowledge, this is the first study that identifies the difference in clonal heterogeneity between UTUC and UCB. Earlier studies had implied that few subclones were identified in bladder cancer, which was is in accordance with our result for UCB (20). DNA adducts of AA induces lesion site structural perturbations and conformational heterogeneity of damaged DNA (40). Moreover, AA exposure leads to suppress DNA repair (41). In this study, we speculated 20.91% harboring AA exposure in UTUC and none in UCB according to rate of AA signature, which may explain the reason why the clonal and subclonal mutation numbers in UTUC were higher than those in UCB. In addtion, it has been demonstrated that high clone number is significantly associated with poor survival in glioblastoma and lung cancer (42, 43). Therefore, we speculated that UTUC had a worse prognosis than UCB, possibly because of its higher number of clones and subclones and higher heterogeneity. However, this inference requires further studies of larger patient cohorts and complete clinical information.

Driver mutations occur in single cells and occur early in the tumor, serving as the most important role in tumorigenesis. In this study, *TP53, PIK3CA*, and *FGFR3* mutations were the candidate oncogene driver genes for UTUC, whereas the candidate oncogene driver gene was *BRCA1* for UCB. The result may imply that the carcinogenic mechanism was different between UTUC and UCB and explain the reason for the differences in clinical characteristics, epidemiology, and molecular mechanisms.

Tumor genomic analysis contributes to guiding the clinical management of cancer patients. For example, the landscape of mutations can guide treatment selection by genes of clinical actionability or biomarkers. Several studies have confirmed that either *DDR*, CNV or TMB were associated with clinical response to checkpoint blockade immunotherapy and patient survival (14, 44). However, there is no systematic comparison of these three factors between UTUC and UBC. In this study, we found that *DDR* gene mutations, CNV number, and TMB were relatively common between UTUC and UCB. This may reflect that patients with UTUC or UCB are equally likely to benefit from immunotherapy based on those markers. Necchi et al. reported that patients with UTUC had a lower median TMB than patients with UCB (5.2 vs 6.9 mut/Mb) (45). Nassar et al. demonstrated that compared to UTUC patients, UCB patients harbored a higher frequency of *DDR* gene mutations (26). One possible explanation is that most Chinese UTUC patients had an exposure history of AA, because exposure to AA would lead to an increase in TMB and *DDR* mutations (46). Multiple studies have shown that DDR gene alterations were associated with higher TMB, which is consistent with our results (26, 47). Notably, Balar et al. reported that patients with UTUC had a higher objective response than UCB (39% vs 17%) (48). Thus, exploring more markers for immunotherapy is an urgent problem to be solved, and existing marker combination analysis may be a new research direction.

In this study, there was no significant difference in the proportion of patients receiving targeted therapy between UTUC and UCB (53.33% vs. 54.79%). *FGFR2/3* mutations were similar between UTUC and UCB (20% vs. 21.91%), which was consistent with Sung who reported 20% of mUC patients treated with platinum-based chemotherapy presented FGFR3 mutations (49). The FDA has approved erdafitinib for patients with urothelial carcinoma whose tumors have susceptible *FGFR3* or *FGFR2* genetic alterations. Those results suggest that only 20% of UC patients can benefit from erdafitinib, and 30% of patients may benefit from cross-indication targeted therapy. Moreover, 50% of patients need to have new targeted drugs developed, or other treatments based on the molecular characteristics.

The present study had several limitations: First, the results should be limited to the number of samples and mutations analyzed in the current study. Second, the results spanned a lengthy time, and patients were scheduled for various therapies according to each patient's condition. Lastly, only targeted DNA sequencing was performed. Integration of genome, transcriptome and proteomic analyses may thus provide further insights into the biology of UC.

Conclusion

Our findings lay the foundation for a deeper understanding of distinct molecular mechanisms and similar treatment opportunities between UTUC and UCB. A comprehensive understanding of the biology of UTUC and UCB is needed to identify new drug targets in order to improve clinical outcomes.

Abbreviations

UC: urothelial carcinoma; UTUC: upper tract urothelial carcinoma; UCB: urothelial carcinoma of the bladder; NGS: next-generation sequencing; AA: aristolochic acid; CNV: copy number variation; TMB: tumor mutational burden; NER: nucleotide excision repair; MMR: Mismatch repair; FA: Fanconi Anemia, HR: homologous recombination; KEGG: KOBAS-Kyoto Encyclopedia of Genes and Genomes; CCF: Cancer Cell Fraction;

Declarations

Ethics approval and consent to participate: This study was approved by the Ethical Committee of Peking University First Hospital. Informed consent was obtained from all participants who understand the content of the experiment and agree to publish the article.

Consent for publication: all participants agree to publish the article.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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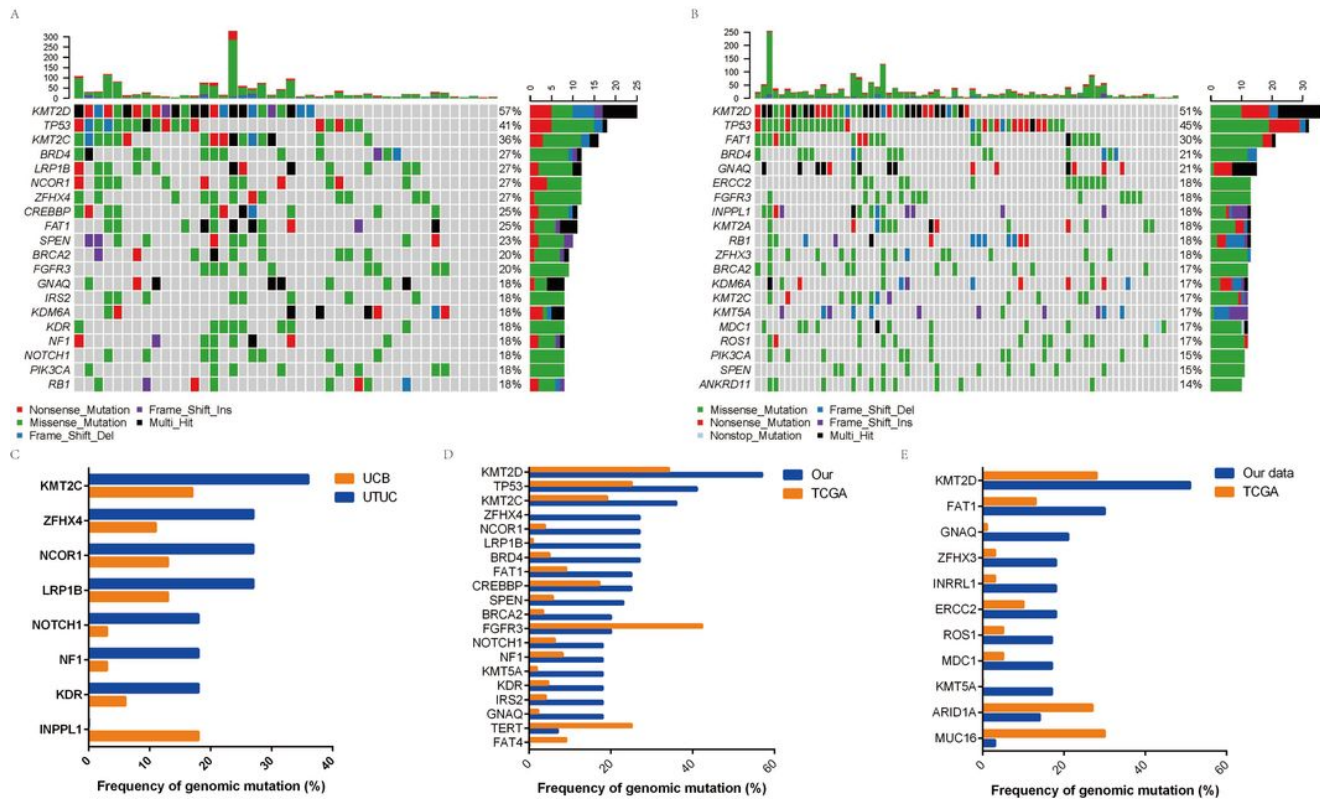
Figures

Figure 1

Landscapes of frequently mutated genes in different locations in UC. The most frequently mutated genes in (A) UTUC, (B) UCB. Names of the top 20 mutated genes (left), frequency of the top 20 mutated genes (right), TMB (above), and mutation type for each sample (bottom). (C) Significant differences in variant allele frequencies for the top 20 genes between UTUC (blue bars) and UCB (red bars) cohorts. (D) Significant difference in the frequency of genomic mutation for the top 20 genes among our UTUC cohort (green bars) and TCGA UTUC cohort (red bars) cohorts. (E) Significant difference in the frequency of genomic mutations for the top 20 genes among our UCB cohort (green bars) and TCGA UCB cohort (red bars) cohorts.
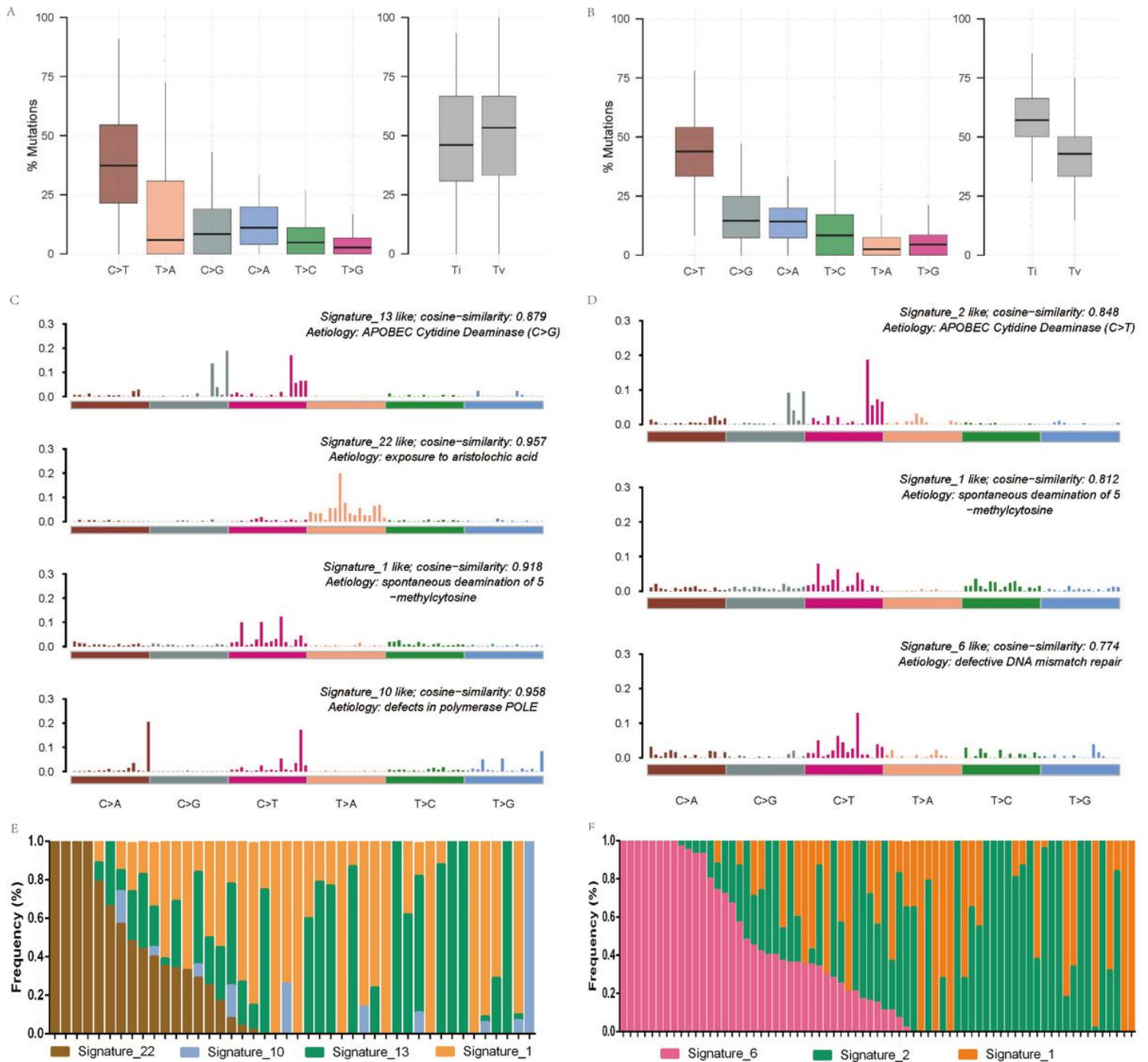
Figure 2

Dominant mutational signatures between UTUC and UCB. (A) The bar plot shows each type of transition or transversion in UTUC cohorts. (B) The bar plot shows each type of transition or transversion in UCB cohorts. (C) Mutational signatures in UTUC cohorts. (D) mutational signatures in UCB cohorts. (E) The box plot shows the ratio of mutational signatures for each patient with UTUC. (F) The box plot shows the ratio of mutational signatures for each patient with UCB.
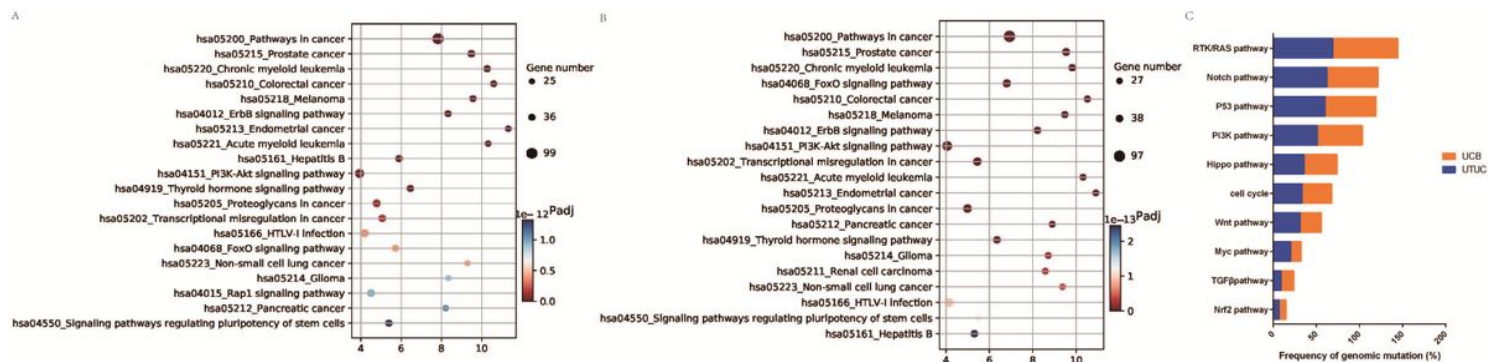
Figure 3

Gene function analysis in both UTUC and UCB. (A) KEGG enrichment for UTUC (B) KEGG enrichment for UCB. (C) Frequency of mutations in oncogenic pathways in both groups.
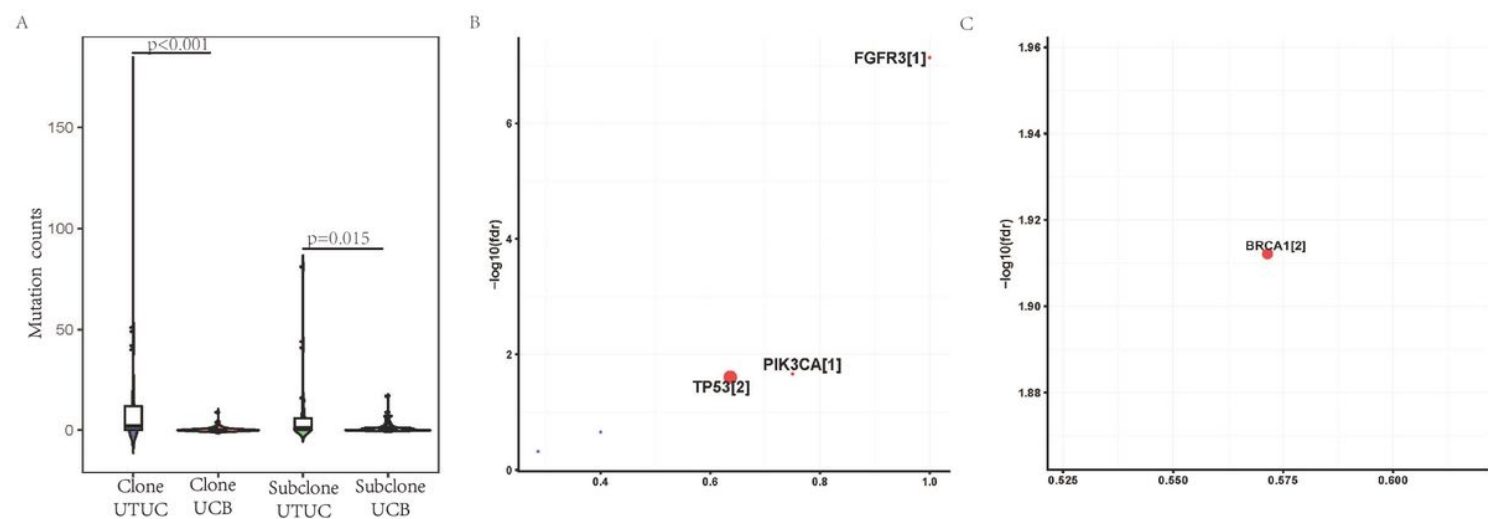

Figure 4

Differences of clone mutation burden and driver gene between UTUC and UCB. (A) Comparison of clonal and subclonal mutation burden between UTUC and UCB. Significance from Wilcoxon rank-sum test is indicated. (B) Identification driver genes in UTUC. (C) Identification driver genes in UCB.
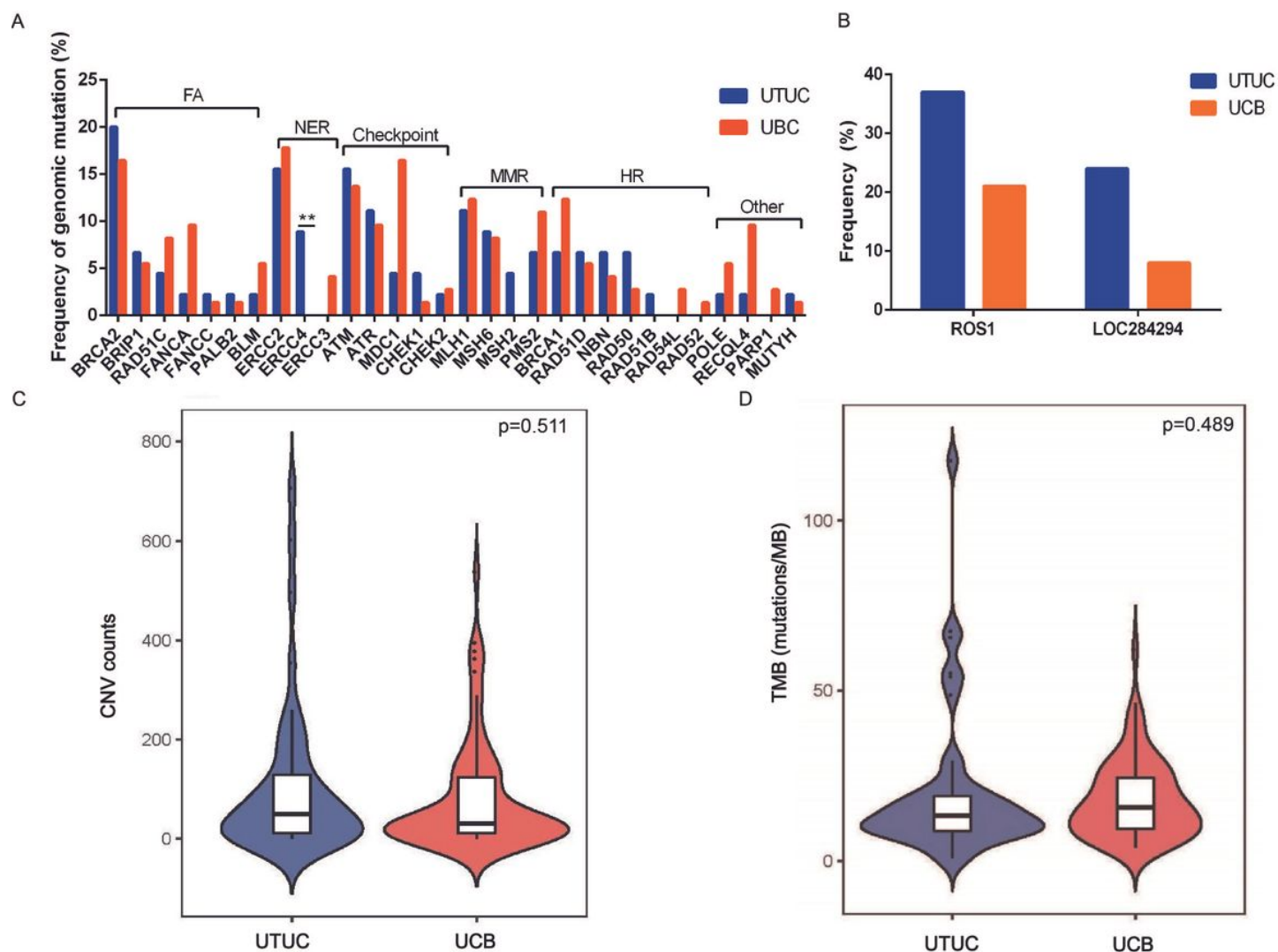
Figure 5

Distribution of markers related to treatment between UTUC and UCB. (A) Distribution of DDR gene alterations. (B) Significant differences in genes in CNV counts for the top 20 genes between UTUC (blue bars) and UCB (red bars) cohorts. (C) differences in CNV counts between UTUC (blue bars) and UCB (red bars) cohorts. (D) differences in TMB between UTUC (blue bars) and UCB (red bars) cohorts.
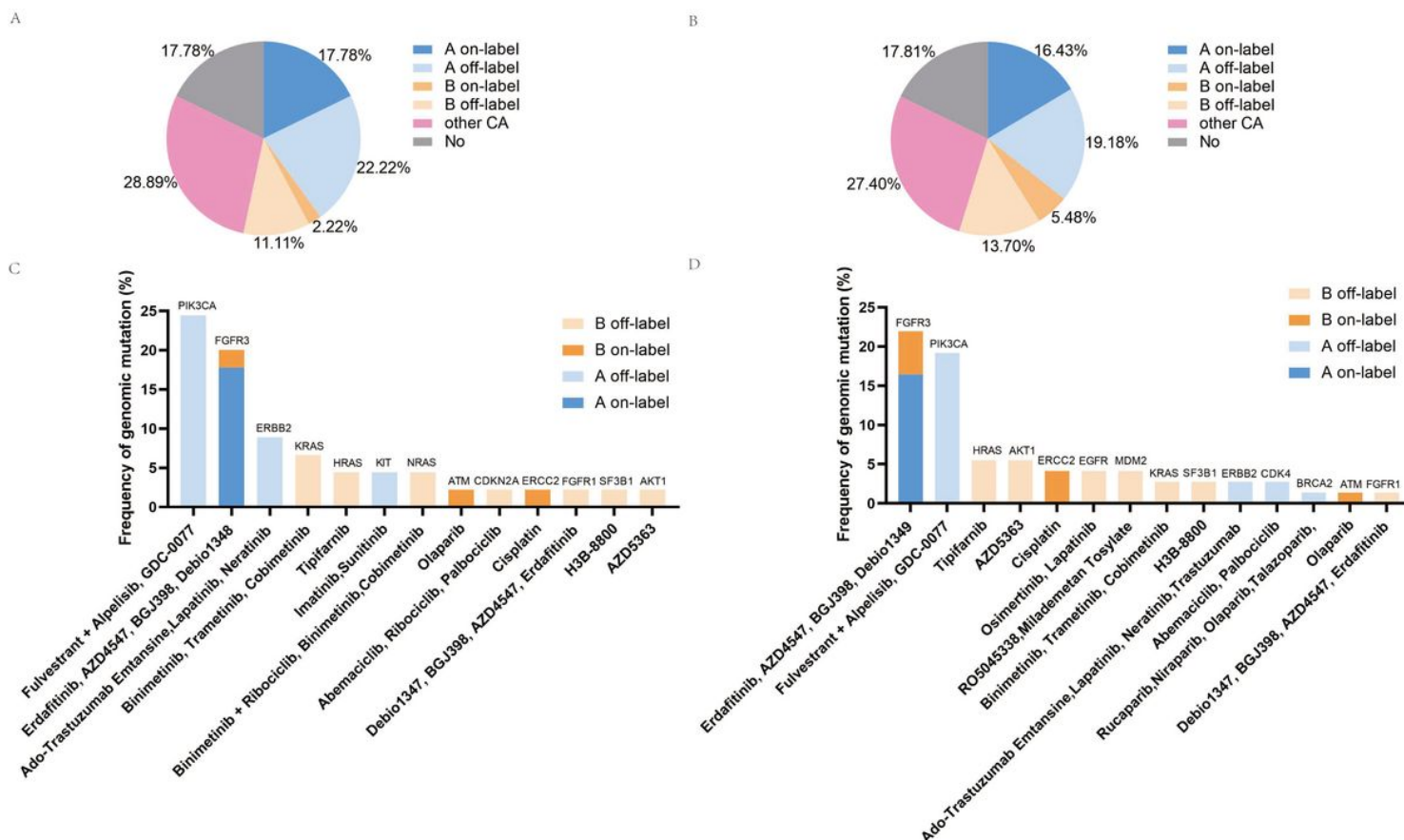
Figure 6

Clinical actionability of somatic alterations in both UTUC and UCB. (A) Distribution of the levels of actionable alterations in UTUC. (B) Distribution of the levels of actionable alterations in UCB. (C) Distribution of targeted genes and drugs in UTUC. (D) Distribution of targeted genes and drugs in UTUC. Level A, which corresponded to level 1 and level 2 in OncoKB, represents the presence of biomarkers with either an approved therapy or guidelines, and level B, which corresponded to level 3 and level 4 in OncoKB, represents biomarkers with strong biological evidence or clinical trials indicating that they are actionable. On-label indicates a treatment registered by federal authorities for bladder cancer, whereas off-label indicates a registration for other tumor types.

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