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Original Article

Distinct molecular signatures of upper tract urothelial carcinoma in Southwestern Taiwan: implications for targeted therapy and disease progression

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Abstract: The incidence of upper tract urothelial carcinoma (UTUC) continues to rise in Southwestern Taiwan, despite a reduction in known environmental carcinogens. This study aimed to characterize the mutational and molecular profiles of UTUC in this high-incidence region and evaluate potential therapeutic targets. We performed next-generation sequencing using the TruSight Oncology 500 panel on 19 formalin-fixed, paraffin-embedded UTUC samples. We analyzed single nucleotide variants (SNVs), insertions/deletions (INDELs), copy number variants (CNVs), microsatellite instability (MSI), and tumor mutational burden (TMB). MSI was stable in all cases, and 42.1% of samples exhibited high TMB (>20 mutations/Mb), often co-occurring with inactivation of TP53, BRCA1, or BRCA2. CNVs were significantly more frequent in advanced-stage UTUC (46.2%) than in early-stage disease (0%). FGFR3 mutations were enriched in early-stage tumors (83.3%), while TP53 mutations predominated in advanced-stage tumors (46.2%). Notably, actionable mutations in PIK3CA, ERBB2, BRCA1, and BRCA2 occurred at higher frequencies than in previously reported Japanese UTUC cohorts. Our findings reveal a distinct molecular signature of UTUC in Southwestern Taiwan, with early- and late-stage tumors showing divergent mutational landscapes. These insights emphasize the importance of molecular stratification in UTUC management and suggest that a broader repertoire of targeted therapies could benefit patients in this high-incidence region.

Keywords: Urinary bladder urothelial cancer, upper tract urothelial cancer, tumor mutation burden, FGFR3, ERBB2, TP53

Introduction

Upper tract urothelial cancer (UTUC) is a subtype of urothelial cancer (UC) that primarily arises in the renal pelvis. UTUC is relatively uncommon in the United States, representing approximately 5% of all UC cases [1, 2]. In contrast, data from the 2020 Taiwan Cancer Registry Annual Report documented 2,410 newly diagnosed cases of urinary bladder urothelial cancer (UBUC) and 1,752 cases of UTUC, indicating that UTUC accounts for nearly 42% of UC cases in Taiwan. A marked sex disparity has been o reported: the age-standardized sex ratio is 2.81 for UBUC but only 0.9 for UTUC. The highest incidence of UTUC occurs in Southwestern Taiwan [3]. Collectively, these epidemiological observations highlight a region-specific public health concern requiring urgent attention. Environmental exposures and dietary toxins have long been implicated in the development of UBUC and UTUC. During the mid-20

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century, prior to widespread implementation of municipal water systems, Southwestern Taiwan was a recognized hotspot for UBUC, primarily due to consumption of arsenic-contaminated deep well water [3, 4]. Following the introduction of municipal water infrastructure, the incidence of UBUC declined in parallel with reductions in black-foot disease prevalence [5, 6]. Another established risk factor is aristolochic acid, a compound historically present in certain herbal preparations for weight loss [7, 8]. Aristolochic acid is nephrotoxic, induces DNA adduct formation, and promotes carcinogenesis within the urinary tract. Although aristolochic acid-containing products were banned in Taiwan in 2003, the incidence of UTUC has continued to rise over the past two decades, while UBUC incidence has declined [9]. These divergent epidemiological trends suggest that aristolochic acid exposure plays only a partial role in UTUC pathogenesis, and additional environmental or dietary risk factors have not yet been identified.

The cornerstone of treatment for non-metastatic UTUC is surgical resection. Radical nephroureterectomy is recommended for high-risk disease, whereas kidney-sparing surgery may be considered for low-risk tumors [10, 11]. Prognosis and recurrence risk depend on tumor number, size, grade, stage, and other pathological features [10, 11]. For advanced or metastatic UTUC, systemic therapy typically involves cisplatin- or platinum-based combination chemotherapy [10-12]. Immunotherapies and targeted therapies are available for selected patients; however, clinical benefit is limited, indicating that additional mechanisms of therapeutic resistance remain to be elucidated. Tumor mutation burden (TMB) emerged as a predictive biomarker for immunotherapy responsiveness in malignancies such as melanoma and non-small-cell lung cancer [13, 14]. Its predictive value in UTUC, however, remains uncertain.

Genomic profiling has revealed widespread alterations in advanced bladder cancer [15]. A comprehensive report demonstrated that 99.7% of analyzed cases harbored at least one genomic alteration, with an average of 6.4 alterations per tumor; 93% of tumors carried at least one clinically actionable alteration [15]. The most frequent included *CDKN2A* (34%), *FGFR3* (21%), *PIK3CA* (20%), and *ERBB2* (17%) [15]. These findings support the potential of genomics to guide targeted therapies in refractory UC.

Over the past decade, UTUC incidence in Taiwan - particularly in Southwestern regions - has risen despite the elimination of known carcinogenic exposures more than 20 years ago. Intriguingly, UTUC occurs more frequently in women, further suggesting a unique and as-yet-uncharacterized etiology. The disproportionately high incidence of UTUC in Taiwan underscores the need for systematic molecular investigation. Comprehensive next-generation sequencing assays may help identify predictive biomarkers and novel therapeutic targets.

In this study, we analyzed UTUC specimens collected in Southwestern Taiwan using a clinically validated tumor mutation panel. Genomic findings were compared with previously reported alterations in UBUC and UTUC, including those from Japanese cohorts. Our results provide insights into the distinct molecular features of UTUC in Taiwan and may serve as a foundation for the development of improved treatment and patient management strategies for refractory cases in high-incidence regions.

Materials and methods

Ethical approval and patient recruitment

This study was approved by the Institutional Review Board of Ditmanson Medical Foundation Chiayi Christian Hospital (approval No. 2020062). All procedures for specimen acquisition and analysis adhered to the principles outlined in the Declaration of Helsinki.

Patients diagnosed with UTUC at Ditmanson Medical Foundation Chiayi Christian Hospital were recruited. The cohort consisted of 19 patients (10 males and 9 females), including 13 with late-stage disease (stage III-IV) and 6 with early-stage disease (stage I-II). All patients underwent surgical resection, and tumor histology was confirmed as carcinoma by board-certified pathologists. Clinical and demographic characteristics are summarized in **Table 1**.

Tissue collection and DNA extraction

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were obtained from the Department of Pathology, Ditmanson Medical

Table 1. Patient characterizes

Characterizes	UTUC early stage (N=6)		UTUC advanced stage (N=13)		*p* value
	No.	%	No.	%	
Age-mean ± sd	75.2 ± 6.20		67.6 ± 8.65		0.064
Sex					
Male	5	83.3	5	38.5	0.062
Female	1	16.7	8	61.5	
Stage					
Stage I	4	66.6	0	0	
Stage II	2	33.3	0	0	
Stage III	0	0	7	53.8	
Stage IV	0	0	6	46.2	
Treatment					
Curative Surgery	6	100	13	100	0.244
Systemic treatment	1	16.7	5	38.5	
Recurrent or Progression					
No	5	83.3	7	53.8	0.678
Within 6 months	1	16.7	4	30.8	
More than one year	0		2	15.4	

Foundation Chia-Yi Christian Hospital. In total, of 11 UBUC and 8 UTUC specimens were included. DNA was extracted using the *GeneRead DNA FFPE Kit* (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. DNA concentration was quantified using the Qubit dsDNA *Hi*gh-Sensitivity *A*ssay (Thermo-Fisher, Waltham, MA USA).

Mutation analysis

Genomic alterations - including single nucleotide variations (SNVs), insertions/deletions (INDELs), microsatellite instability (MSI), and copy number variants (CNVs) - were analyzed using the *TruSight Oncology 500* (TSO500) panell (Illumina, San Diego, CA, USA). Library preparation was performed according to the manufacturer's protocol. Briefly, purified DNA was enzymatically fragmented, size-selected using the *AMPure XP magnetic system* (Beckman Coulter, Brea, CA, USA), and quality assessed via capillary electrophoresis with the *D1000 ScreenTape Assay* on a *TapeStaton 2200 analyzer* (Agilent Technologies, Santa Clara, CA, USA).

Fragmented DNA underwent end-repair, A-tailing, and adaptor ligation with unique molecular indices, followed by PCR amplification. Target enrichment was achieved through hybridization to biotin-labeled probes at 57°C for 18-24 h, capture with streptavidin-coated magnetic beads, and sequential washing, elution, and rehybridization enrichment steps. The final sequencing-ready libraries were again quality-checked and sequenced on an *Illumina NovaSeq 6000* platform.

Bioinformatic analysis

Gene copy number variations were analyzed using the *TSO500 assay*, a comprehensive panel designed to assess CNVs in genes associated with oncogenesis (Figures S1, S2). The genes were selected based on their relevance to UTUC and their inclusion in the TruSight panel, which covers key genes involved in tumorigenesis, DNA repair mechanisms, and immune evasion.

Raw sequencing reads were trimmed to 100 bp, and processed using the *TSO500 Local App 2.2*. Reads were mapped to the human reference genome (hg19), collapsed using unique molecular indices (UMIs), and remapped. Variants were annotated to generate variant call format (VCF) file, which were subsequently visualized and classified using the *Illumina BaseSpace Variant Interpreter*.

Microsatellite sites were assessed, and MSI status was reported as the percentage of unstable loci those detected. To filter germline

Table 2. Demographic data of the study cohort

No.	Early Stage UTUC						Advanced Stage UTUC												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Gender	M	M	M	F	M	M	M	M	F	F	F	F	M	F	F	F	M	M	F
Stage	1I	I	I	I	II	II	III	III	III	III	III	III	III	IV	IV	IV	IV	IV	IV
TMB (nb)	5.5	62	29.8	87.7	4.7	7.2	0	3.2	11.1	12.5	26.1	33.4	43.5	5.6	5.9	8.6	11.8	29	133.7
MSI (%)	4.7	3.2	2.4	3.2	7.6	4.5	4.0	4.2	6.5	3.2	2.5	2.4	4.6	3.1	1.7	1.7	2.7	3.6	1.7
Recurrent stats (yes/no)	No	No	No	No	No	Yes	No	No	No	No	Yes	Yes	No						
Disease Status (CR/SD/PD)	CR	CR	CR	CR	CR	PD	CR	CR	CR	CR	PD	PD	CR	PD	PD	SD	PD	SD	PD

CR, complete remission; No., number; SD, stable disease; PD, progress disease. TMB and MSI were calculated from the TSO500 sequencing data by using the analysis pipeline TSO500 local app v.2.2.

and common population variants, annotated variants were compared against the *GnomAD Exome*, *GnomAD Genome*, and *1000 Genomes* databases. TMB was calculated as the number of somatic mutations per megabase after excluding variants with a variant allele frequency (VAF) <5%. Annotation of the variants was performed using the ClinVar database, which categorizes variants based on clinical significance and prior reports.

Statistical analysis

Statistical analysis was performed using a two-sample t-test to compare continuous variables between two groups. A *p*-value of <0.05 was considered statistically significant.

Results

Characteristics of patients with UTUC

The sectioned FFPE specimens were analyzed using the Illumina Oncology 500 DNA panel. The analysis pipeline enabled the identification of SNVs and INDELs as well as the calculation of CNV, TMB, and MSI. The clinical characteristics, TMB scores, and MSI status are summarized in **Table 2**. All patients displayed stable microsatellite sequences, consistent with previous reports (94.7%) [16]. A high TMB (TMB >20) was found in 8/19 (42.1%) patients, occurring across both early and advanced stages. igh TMB did not correlate with sex, age, or stage, suggesting High TMB did not correlated with sex, age, or cancer stage, distinct oncogenic mechanisms between high- and low-TMB UTUC.

Gene copy number variations in patients with UTUC

The TSO500 sequencing panel and associated analysis pipeline were used to assess focal amplifications in preselected genes. The results are summarized in **Tables 3** and S1. CNV events were identified in six cases of advanced stage UTUC, while none were observed in early stage UTUC. Specifically, patients #11 (Stage III) and #12 (Stage III) exhibited CNV events with amplification of *CCND1*, *FGF19*, *FGFR4*, and *FGFR3*. These genes are located on the long arm of chromosome 11, suggesting the possibility of structural variation within this region. Patient #15 (Stage IV) demonstrated an additional copy amplification of *ERBB2* on chromosome 17. Similarly, patient #19 (Stage IV) exhibited amplification of *FGFR4*, but without concurrent amplification of neighboring *FGF19* and *FGFR3*. In total, amplification of the chromosome 11 q13 region was observed in patients #11 (Stage III) and #12 (Stage III), and #19 (Stage IV). The role of this CNV event in UTUC carcinogenesis or progression warrants further investigation. In patient #8 (Stage III), simultaneous amplifications of *NRAS*, *RAF1*, and *FGFR1* were detected. Patient #12 (Stage III) demonstrated amplification of both *ERBB2* and *CCND1*. Lastly, patient #13 (Stage III) exhibited moderate MYC amplification. Overall, a higher percentage of patients with advanced-stage UTUC (6 of 13, 46.2%) exhibited CNV events compared to those with early-stage UTUC (0 of 6, 0%).

Mutations in primary therapeutic target genes

Variants annotated as pathogenic and likely pathogenic in ClinVar were retained for subsequent comparisons between the early-stage and the advanced-stage UTUC. Some of the pathogenic variants identified in this study were target variants for FDA-approved therapies and were included in the target lists of corresponding companion diagnostics. These variants were located in genes such as *FGFR3*, *BRCA1, BRCA2*, *ERBB2*, and *PIK3CA* (**Tables 4** and S1).

Table 3. Copy number variant events identified in UTUC patients

No.		Early Stage UTUC						Advanced Stage UTUC												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Stage		I	I	I	I	II	II	III	III	III	III	III	III	III	IV	IV	IV	IV	IV	IV
TMB		5.5	62	29.8	87.7	4.7	7.2	0	3.2	14	12.5	26.1	42	43.5	5.6	5.9	8.6	11.8	29	133.7
Gene	Chr																			
NRAS	1								5.9											
RAF1	3								15.9											
MYC	8													4.7						
FGFR1	8								24.2											
CCND1	11											8.5	4.9							
FGF19	11											9.4	5.9							
FGFR4	11											10.1	6.1							4.6
FGFR3	11											10.6	7.7							
ERBB2	17												9.2			4.7				

TMB was calculated as the total number of mutations detected per megabase (Mb) of genomic DNA. Specifically, TMB was determined by dividing the total number of mutations by the size of the exonic region analyzed (in megabases). Data for TMB calculation were derived directly from the Illumina TruSight Oncology 500 sequencing platform. No., number.

The most frequently mutated gene was *FGFR3*, with the p.(Ser249Cys) variant detected in 5 out of 11 cases. Additionally, the p.(Ser373Cys) variant in *FGFR3* was identified in patient #4 (Stage I). Notably, *FGFR3* mutations were more prevalent in early-stage UTUC than in advanced-stage UTUC (83.3% vs. 8.7%, respectively). Other common mutations included the *ERBB2* mutation and *PIK3CA* activation mutations, each found in two cases (10.5% of patients).

Pathogenic variants in tumor suppressors

In addition to mutations targeted by therapies, this study also identified loss-of-function mutations in tumor suppressor genes. The most common of these were pathogenic and likely pathogenic variants of *TP53*. Six out of the 13 advanced-stage UTUC cases (46.2%) harbored *TP53* mutations, while only one out of six early-stage UTUC cases (16.7%) exhibited a *TP53* mutation (**Tables 5** and S1). A premature termination mutation in *TSC1* was identified in one case, and a *PTEN* mutation was detected in another.

In addition to *TP53*, other loss-of-function mutations in cancer suppressor genes, including *BRCA1/BRCA2*, *TSC1* and *PTEN*, were observed in this study. When pooling these cases, we found that inactivation of these five tumor suppressor genes was strongly correlated with a high tumor mutational burden (**Figure 1**).

Discussion

The incidence of UTUC in Taiwan has been steadily increasing over the past decade, particularly in certain southwestern townships. This study identifies genomic aberrations specifically associated with UTUC, highlighting variations in pathogenic variants of tumor suppressor genes between early and advanced stages of the disease. A strong correlation was found between the inactivation of these genes and a high TMB. In patients with advanced-stage UTUC, there was an increase in the copy numbers of several genes, including *NRAS*, *RAF1*, *MYC*, *FGFR1*, *CCND1*, *FGF19*, *FGFR4*, *FGFR3*, *ERBB2*, and *CCND1*, which were not observed in early-stage patients. Additionally, no patients exhibited MSI, suggesting that DNA mismatch repair mechanisms remain functional in both early and advanced UTUC cases, consistent with findings from previous genomic studies of UBUC and UTUC [15-17].

A large genomic analysis study of UTUC in Japan identified the most common mutations as *TP53* (37.3%), *FGFR3* (35.2%), and *RAS* (15.1%) [17]. The *TP53* mutation was predominantly found in invasive UTUC (80% of 75 cases), while *FGFR3* was more commonly associated with non-invasive UTUC (75.7% of 70 cases). In our study of UTUC samples from Southwestern Taiwan, the most frequent mutation was *TP53* (36.8%), which was primarily found in advanced-stages cases (85.7% of 7 cases). The second most

Table 4. Therapy-targeted pathogenic variants identified in the study cohort

No.	Early Stage UTUC						Advanced Stage UTUC												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Stage	I	I	I	I	II	II	III	III	III	III	III	III	III	IV	IV	IV	IV	IV	IV
TMB	5.5	62	29.8	87.7	4.7	7.2	0	3.2	14	12.5	26.1	42	43.5	5.6	5.9	8.6	11.8	29	133.7
Gene																			
FGFR3 (%) c.1117A>T				43.1															
FGFR3 (%) C746C>G	29.7	19.0			43.2	87.3										52.5			
BRCA1 (%) C1138C>T		22.5																	
BRCA2 (%) C771_775del										24.1									
PIK3CA (%) C1624G>A		36.5																	
PIK3CA (%) C1633G>A																	35.1		
ERBB2 (%) C929C>T												9.20			4.70				

TMB was calculated as the total number of mutations detected per megabase (Mb) of genomic DNA. Specifically, TMB was determined by dividing the total number of mutations by the size of the exonic region analyzed (in megabases). Data for TMB calculation were derived directly from the Illumina TruSight Oncology 500 sequencing platform. No., number.

Table 5. Pathogenic variants in tumor suppressors identified in the cohort

No.		Early Stage UTUC						Advanced Stage UTUC												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Stage		I	I	I	I	II	II	III	III	III	III	III	III	III	IV	IV	IV	IV	IV	IV
TMB		5.5	62	29.8	87.7	4.7	7.2	0	3.2	14	12.5	26.1	42	43.5	5.6	5.9	8.6	11.8	29	133.7
Gene (%)	HGVSC																			
TP53	c.193A>T																			14.8
TP53	c.326T>G											54.9								
TP53	c.452C>G										18.5									
TP53	c.614A>T													64.9						
TP53	c.743G>A								39.0											
TP53	c.840A>T												39.2							
TP53	c.854A>T					42.6														
TSC1	c.866C>G				20.9															
PTEN	c.103A>G																		17.1	
BRCA1	c.1138C>T		22.5																	
BRCA2	c.771_775del										24.1									

TMB was calculated as the total number of mutations detected per megabase (Mb) of genomic DNA. Specifically, TMB was determined by dividing the total number of mutations by the size of the exonic region analyzed (in megabases). Data for TMB calculation were derived directly from the Illumina TruSight Oncology 500 sequencing platform. No., number.

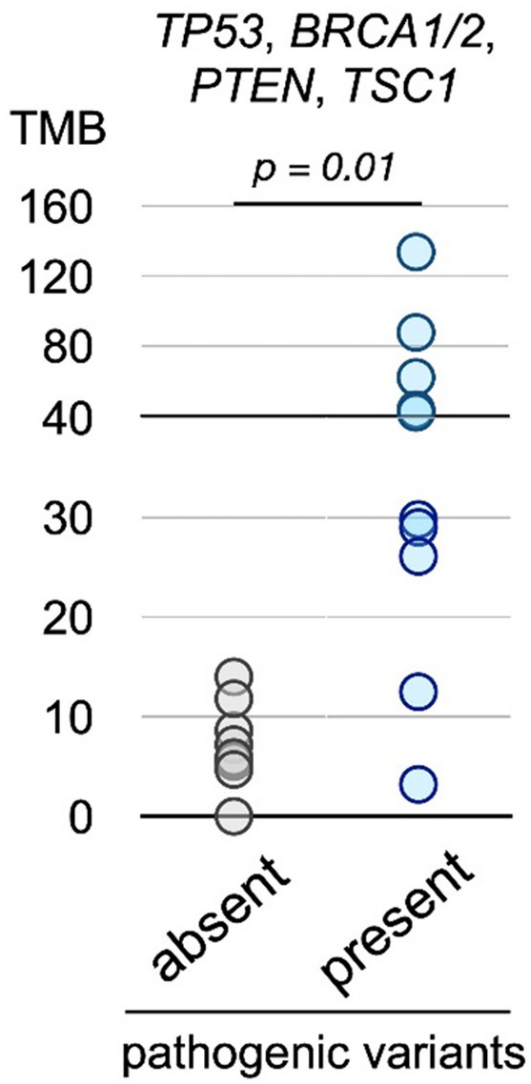

Figure 1. Pathogenic variants in tumor suppressors are correlated with higher TMB.

common mutation was *FGFR3* (31.6%), which was predominantly observed in early-stage cases (83.3% of 6 cases).

In addition to mutations in FGFR3, ERBB2, and PIK3CA, we also observed one instance each of BRCA1 and BRCA2 loss-of-function mutations, suggesting a potential defect in homologous recombination repair. This finding warrants further investigation to assess its clinical relevance (**Table 4**). Other mutations commonly found in Japanese UTUC, such as *CCND1*, *RAS*, *MET*, were also detected in our samples, though at a lower frequency (5-10% of cases, respectively). Additionally, we identified genetic alterations that are commonly associated with UBUC, including PIK3CA, ERBB2, BRCA1, and BRCA2. These alterations occurred more frequently in our UTUC samples than previously reported, with an incidence of 10.5% in our cohort [15]. These genetic findings may help explain the higher incidence of UTUC in Southwestern Taiwan compared to other countries. The CNV analysis revealed that a higher proportion of advanced-stage UTUC cases exhibited at least one CNV event compared to early-stage UBUC cases. Specifically, CNV events were more prevalent in advanced-stage (stage III and IV) disease, suggesting that copy number gains may represent a later event in oncogenesis and could be associated with increased aggressiveness and metastasis. Notably, two patients with UTUC exhibited ERBB2 amplification, a known marker of poor outcomes in UTUC [18]. Although anti-HER2 therapy has been reported to be ineffective in unselected patient populations, it may offer therapeutic benefits in HER2-positive or *ERBB2*-amplified patients [19]. In our study, six patients exhibited the *FGFR3* activation mutation, with the majority of these cases being early-stage UTUC. This result is consistent with the Japan study by Fujii et al. [17], who classified UTUC into five mutation subtypes: hypermutated, *TP53/MDM2, RAS, FGFR3*, and triple-negative. In their cohort, 35% of patients with UTUC carried *FGFR* mutations, with *FGFR3* mutations being predominantly associated with non-invasive UTUC (75.7%) [17]. Clinically, Erdafitinib is an FDA-approved targeted therapy for *FGFR2/3*-altered metastatic urothelial cancer [20]. While non-invasive or early-stage UTUC can often be cured by surgical treatment, the *FGFR3* mutation may have clinical significance for adjuvant therapy. A phase 3 study is currently underway to evaluate the therapeutic effects of anti-*FGRF* agents in urothelial carcinoma [21].

In our study, *BRCA1* and *BRCA2* mutations were identified in two patients with advanced-stage UTUC. The use of PARP inhibitors, either alone or in combination with standard chemotherapy, has been evaluated in various clinical trials [22]. The ATLANTIS trial demonstrated that PARP inhibitors [23] are effective in extending progression-free survival in patients with metastatic urothelial carcinoma when used as maintenance therapy. Additionally, two patients with UTUC were found to have targetable *PIK3CA* mutations, specifically p. (Glu542Lys).

PI3K inhibitors have been approved for the treatment of metastatic estrogen receptor-positive breast cancer with *PIK3CA* hotspot mutations [24, 25]. However, the efficacy of PI3K inhibitors in metastatic urothelial carcinoma remains unestablished. Furthermore, two additional patients carried an *ERBB2* mutation. This mutation, located in the HER2 extracellular domain, promotes the formation and activation of the HER2-EGFR heterodimer [26]. An *in vitro* study demonstrated that anti-HER2 treatment can inhibit the growth of UC cell lines in a xenograft mice model, suggesting potential clinical benefits [27]. Although PARP inhibitors, anti-HER2 treatment, and PI3K inhibitors have not yet been approved by the FDA for the treatment of UBUC and UTUC, case studies suggest that these therapies may offer clinical benefits to patients harboring the targeted mutations.

In our study, as well as in others, *TP53* mutations were the most frequently occurring loss-of-function mutations in tumor suppressor genes. These *TP53* mutations were identified in seven UTUC patients; however, effective treatment options for this group remain limited. Additionally, one patient was found to harbor a *PTEN* mutation, which was annotated as either likely pathogenic or pathogenic. *PTEN* inactivation mutations are commonly observed in various solid tumors and contribute to increased downstream activity of the mTOR pathway. Another patient exhibited a *TSC1* premature termination mutation. *TSC1* is a tumor suppressor that inhibits mTOR activity, and activation of the mTOR pathway has been implicated as a frequent event in urothelial carcinoma [28, 29]. These findings are consistent with previous reports, suggesting that increased mTOR activity may play a key oncogenic mechanism about development of urothelial carcinoma.

In our results, therapy-directed mutations were independent of the increase in TMB. However, inactivation of tumor suppressor genes was strongly correlation with high TMB (**Figure 1**). Both BRCA1/2 and TP53 are involved in response pathways initiated by double-stranded DNA breaks (DSBs). Loss-of-function mutations in these DSB repair pathways could contribute to the accumulation of mutations in the genome. On the other hand, TSC1 and PTEN negatively regulate the activity of the mTOR complex. While mTOR promotes cell growth, its direct role in pathways leading to increased genome mutations remains unclear. Whether increased mTOR activity is merely correlational or causative in TMB accumulation requires further investigation.

Systemic chemotherapy, including gemcitabine, cisplatin, carboplatin, doxorubicin, methotrexate, and vinblastine, is commonly used to treat locally advanced or metastatic urothelial carcinoma. For patients unable to tolerate cisplatin or platinum-based therapies, carboplatin-gemcitabine is an alternative regimen [10, 11]. Additionally, immune checkpoint inhibitors such as pembrolizumab, nivolumab, and avelumab are widely used [10, 11]. Pembrolizumab is recommended for those who cannot undergo chemotherapy due to intolerance [30]. Atezolizumab is used as adjuvant therapy for PD-L1 positive tumors [31], while avelumab serves as maintenance therapy for patients without disease progression following chemotherapy [32]. These inhibitors are approved for treating urothelial carcinoma that has progressed during or after platinum-based chemotherapy, or within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy, regardless of PD-L1 expression levels [33]. For patients who do not respond to or fail immunotherapy, second-line erdafitinib has shown positive clinical responses in approximately one-third of patients with *FGFR2/FGFR3* variants or *FGFR3* fusions [34, 35]. Other second-line treatment options include enfortumab vedotin, an antibody-drug conjugate targeting nectin-4 [36, 37], and vinflunine, a microtubule inhibitor [38]. For patients with locally advanced or metastatic urothelial carcinoma who do not respond to these treatments, we recommend comprehensive tumor mutation panel examinations using next-generation sequencing. This approach may help identify potential therapeutic agents for disease control.

Despite the limited sample size in this study, the results revealed significant differences in oncogenic and actionable mutations between early-stage and advanced-stage UTUC. This finding suggests that the molecular signature and underlying oncogenic mechanisms of UTUC may differ from those typically observed in UBUC or in UTUC from other geographic regions. Although the precise etiological mechanisms underlying UTUC remain unclear, it is possible

that some causative mutations were not captured by the panel used in this study. Expanding the scope of our research to include additional somatic mutations, as well as abnormal RNA expression and fusion events, may prove critical. Further investigation is warranted to elucidate the underlying etiological mechanisms and identify potential therapeutic approaches for patients with UTUC from the hotspot region of Taiwan, particularly Southwestern Taiwan.

In conclusions, only a subset of patients benefits from immunotherapy and targeted therapies, suggesting that other molecular mechanisms may contribute to treatment resistance. Our study found that *FGFR3* mutations are more prevalent in early-stage UTUC than in advanced-stage disease, while *TP53* mutations are more common in advanced UTUC - a pattern that diverges from urothelial carcinoma of the bladder. Additionally, we observed a higher incidence of genetic alterations such as *PIK3CA*, *ERBB2*, *BRCA1*, and *BRCA2* compared to the Japanese UTUC study. While these findings could inform treatment strategies and prevention efforts for UTUC, more extensive studies are needed for further investigation these molecular mechanisms and their clinical implications.

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Disclosure of conflict of interest

None.

Abbreviations

CNVs, copy number variants; DSBs, double-stranded DNA breaks; FFPE, formalin-fixed, paraffin-embedded; INDELs, insertions/deletions; MSI, microsatellite instability; SNVs, single nucleotide variations; TMB, tumor mutation burden; VAF, variant allele frequency; VCF, variant call format; UBUC, urinary bladder urothelial cancer; UC, urothelial cancer; UMI, unique molecular index; UTUC, upper tract urothelial cancer.

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ABL1	BRD4	CUX1	FAM175A	GATA6	IGF1	MAP3K13	NOTCH4	POLE	RPTOR	TAF1
ABL2	BRIP1	CXCR4	FAM46C	GEN1	IGF1R	MAP3K14	NPM1	PPARG	RUNX1	TBX3
ACVR1	BTG1	CYLD	FANCA	GID4	IGF2	MAP3K4	NRAS	PPM1D	RUNX1T1	TCEB1
ACVR1B	BTK	DAXX	FANCC	GLI1	IKBKE	MAPK1	NRG1	PPP2R1A	RYBP	TCF3
AKT1	C11ORF30	DCUN1D1	FANCD2	GNA11	IKZF1	MAPK3	NSD1	PPP2R2A	SDHA	TCF7L2
AKT2	CALR	DDR2	FANCE	GNA13	IL10	MAX	NTRK1	PPP6C	SDHAF2	TERC
AKT3	CARD11	DDX41	FANCF	GNAQ	IL7R	MCL1	NTRK2	PRDM1	SDHB	TERT
ALK	CASP8	DHX15	FANCG	GNAS	INHA	MDC1	NTRK3	PREX2	SDHC	TET1
ALOX12B	CBFB	DICER1	FANCI	GPR124	INHBA	MDM2	NUP93	PRKAR1A	SDHD	TET2
ANKRD11	CBL	DIS3	FANCL	GPS2	INPP4A	MDM4	NUTM1	PRKC1	SETBP1	TET3
ANKRD26	CCND1	DNAJB1	FAS	GREM1	INPP4B	MED12	PAK1	PRKDC	SETD2	TFRC
APC	CCND2	DNMT1	FAT1	GRIN2A	INSR	MEF2B	PAK3	PRSS8	SF3B1	TGFBR1
AR	CCND3	DNMT3A	FBXW7	GRM3	ARF2	MEN1	PAK7	PTCH1	SH2B3	TGFBR2
ARAF	CCNE1	DNMT3B	FGF1	GSK3B	IRF4	MET	PALB2	PTEN	SH2D1A	TMEM127
ARFRP1	CD274	DOT1L	FGF10	H3F3A	IRS1	MGA	PARK2	PTPN11	SHQ1	TMPRSS2
ARID1A	CD276	E2F3	FGF14	H3F3B	IRS2	MITF	PARP1	PTPRD	SLIT2	TNFAIP3
ARID1B	CD74	EED	FGF19	H3F3C	JAK1	MLH1	PAX3	PTPRS	SLX4	TNFRSF14
ARID2	CD79A	EGFL7	FGF2	HGF	JAK2	MLL	PAX5	PTPRT	SMAD2	TOP1
ARID5B	CD79B	EGFR	FGF23	HIST1H1C	JAK3	MLLT3	PAX7	QKI	SMAD3	TOP2A
ASXL1	CDC73	EIF1AX	FGF3	HIST1H2BD	JUN	MPL	PAX8	RAB35	SMAD4	TP53
ASXL2	CDH1	EIF4A2	FGF4	HIST1H3A	KAT6A	MRE11A	PBRM1	RAC1	SMARCA4	TP63
ATM	CDK12	EIF4E	FGF5	HIST1H3B	KDM5A	MSH2	PDCD1	RAD21	SMARCB1	TRAF2
ATR	CDK4	EML4	FGF6	HIST1H3C	KDM5C	MSH3	PDCD1LG2	RAD50	SMARCD1	TRAF7
ATRX	CDK6	EP300	FGF7	HIST1H3D	KDM6A	MSH6	PDGFRA	RAD51	SMC1A	TSC1
AURKA	CDK8	EPCAM	FGF8	HIST1H3E	KDR	MST1	PDGRFB	RAD51B	SMC3	TSC2
AURKB	CDKN1A	EPHA3	FGF9	HIST1H3F	KEAP1	MST1R	PDK1	RAD51C	SMO	TSHR
AXIN1	CDKN1B	EPHA5	FGFR1	HIST1H3G	KEL	MTOR	PDPK1	RAD51D	SNCAIP	U2AF1
AXIN2	CDKN2A	EPHA7	FGFR2	HIST1H3H	KIF5B	MUTYH	PGR	RAD52	SOCS1	VEGFA
AXL	CDKN2B	EPHB1	FGFR3	HIST1H3I	KIT	MYB	PHF6	RAD54L	SOX10	VHL
B2M	CDKN2C	ERBB2	FGFR4	HIST1H3J	KLF4	MYC	PHOX2B	RAF1	SOX17	VTCN1
BAP1	CEBPA	ERBB3	FH	HIST2H3A	KLHL6	MYCL1	PIK3C2B	RANBP2	SOX2	WISP3
BARD1	CENPA	ERBB4	FLCN	HIST2H3C	KMT2B	MYCN	PIK3C2G	RARA	SOX9	WT1
BBC3	CHD2	ERCC1	FLI1	HIST2H3D	KMT2C	MYD88	PIK3C3	RASA1	SPEN	XIAP
BCL10	CHD4	ERCC2	FLT1	HIST3H3	KMT2D	MYOD1	PIK3CA	RB1	SPOP	XPO1
BCL2	CHEK1	ERCC3	FLT3	HLA-A	KRAS	NAB2	PIK3CB	RBM10	SPTA1	XRCC2
BCL2L1	CHEK2	ERCC4	FLT4	HLA-B	LAMP1	NBN	PIK3CD	RECQL4	SRC	YAP1
BCL2L11	CIC	ERCC5	FOXA1	HLA-C	LATS1	NCOA3	PIK3CG	REL	SRSF2	YES1
BCL2L2	CREBBP	ERG	FOXL2	HNF1A	LATS2	NCOR1	PIK3R1	RET	STAG1	ZBTB2
BCL6	CRKL	ERRFI1	FOXO1	HNRNPK	LMO1	NEGR1	PIK3R2	RFWD2	STAG2	ZBTB7A
BCOR	CRLF2	ESR1	FOXP1	HOXB13	LRP1B	NF1	PIK3R3	RHEB	STAT3	ZFHX3
BCORL1	CSF1R	ETS1	FRS2	HRAS	LYN	NF2	PIM1	RHOA	STAT4	ZNF217
BCR	CSF3R	ETV1	FUBP1	HSD3B1	LZTR1	NFE2L2	PLCG2	RICTOR	STAT5A	ZNF703
BIRC3	CSNK1A1	ETV4	FYN	HSP90AA1	MAGI2	NFKBIA	PLK2	RIT1	STAT5B	ZRSR2
BLM	CTCF	ETV5	GABRA6	ICOSLG	MALT1	NKX2-1	PMAIP1	RNF43	STK11	
BMPR1A	CTLA4	ETV6	GATA1	ID3	MAP2K1	NKX3-1	PMS1	ROS1	STK40	
BRAF	CTNNA1	EWSR1	GATA2	IDH1	MAP2K2	NOTCH1	PMS2	RPS6KA4	SUFU	
BRCA1	CTNNB1	EZH2	GATA3	IDH2	MAP2K4	NOTCH2	PNRC1	RPS6KB1	SUZ12	
BRCA2	CUL3	FAM123B	GATA4	IFNGR1	MAP3K1	NOTCH3	POLD1	RPS6KB2	SYK	

Content shaded in grey is analyzed for CNV detction.

Figure S1. The list of target genes covered by Illumina TruSight Oncology 500 assay.

ABL1	*BCL2*	*CSF1R*	*ESR1*	*EWSR1*	*FLI1*	*KIF5B*	*MSH2*	*NRG1*	*PAX7*	*RAF1*
AKT3	*BRAF*	*EGFR*	*ETS1*	*FGFR1*	*FLT1*	*KIT*	*MYC*	*NTRK1*	*PDGFRA*	*RET*
ALK	*BRCA1*	*EML4*	*ETV1*	*FGFR2*	*FLT3*	*MET*	*NOTCH1*	*NTRK2*	*PDGFRB*	*ROS1*
AR	*BRCA2*	*ERBB2*	*ETV4*	*FGFR3*	*JAK2*	*MLL*	*NOTCH2*	*NTRK3*	*PIK3CA*	*RPS6KB1*
AXL	*CDK4*	*ERG*	*ETV5*	*FGFR4*	*KDR*	*MLLT3*	*NOTCH3*	*PAX3*	*PPARG*	*TMPRSS2*

All genes listed are assessed for known and novel fusions. In addition, the content shaded in grey is analyzed for splice variants.

Figure S2. The list of fusion events covered by Illumina TruSight Oncology 500 assay.