Gender Disparities in Clinical Outcomes of Urothelial Carcinoma Linked to X Chromosome Gene *KDM6A* Mutation

Running title: *KDM6A* Linked Gender Disparities in UC

Supplementary Materials

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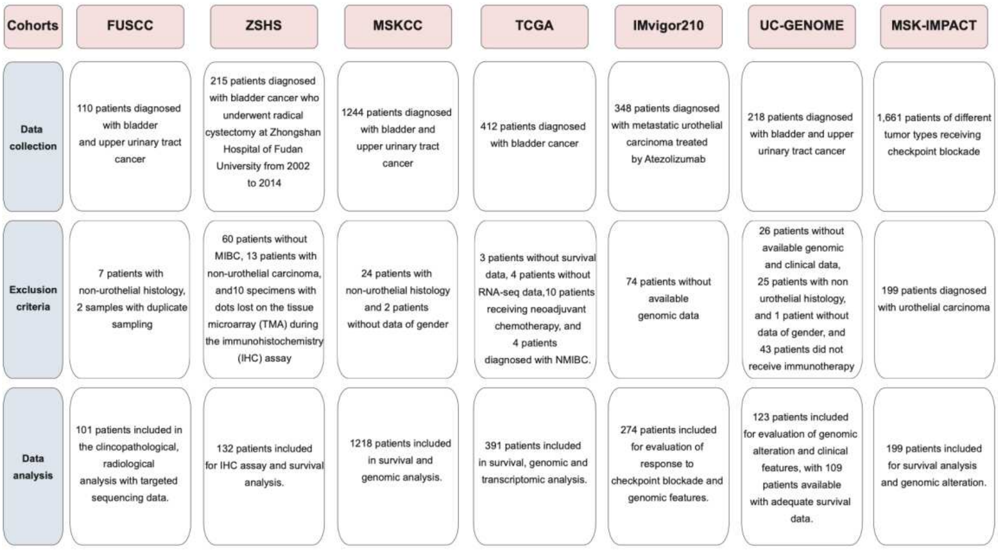
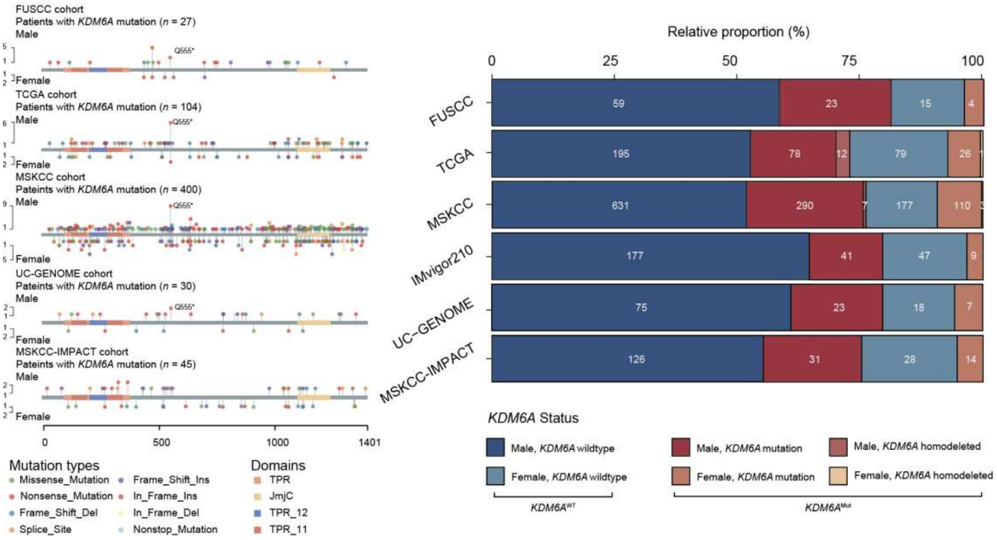
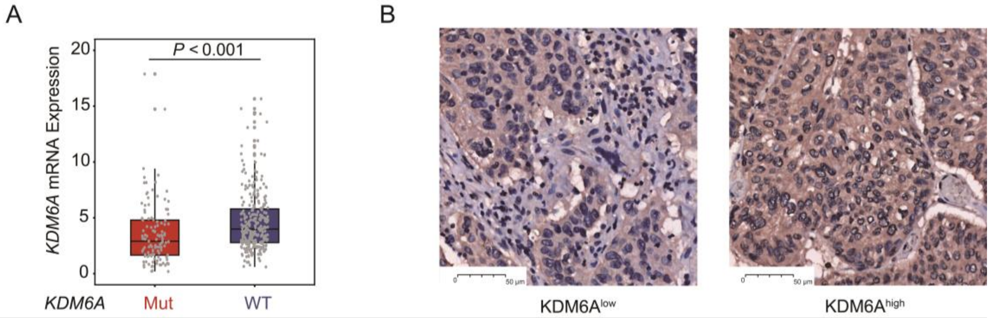


Cohorts	FUSCC	ZSHS	MSKCC	TCGA	IMvigor210	UC-GENOME	MSK-IMPACT
Data collection	110 patients diagnosed with bladder and upper urinary tract cancer	215 patients diagnosed with bladder cancer who underwent radical cystectomy at Zhongshan Hospital of Fudan University from 2002 to 2014	1244 patients diagnosed with bladder and upper urinary tract cancer	412 patients diagnosed with bladder cancer	348 patients diagnosed with metastatic urothelial carcinoma treated by Atezolizumab	218 patients diagnosed with bladder and upper urinary tract cancer	1,661 patients of different tumor types receiving checkpoint blockade
Exclusion criteria	7 patients with non-urothelial histology, 2 samples with duplicate sampling	60 patients without MIBC, 13 patients with non-urothelial carcinoma, and10 specimens with dots lost on the tissue microarray (TMA) during the immunohistochemistry (IHC) assay	24 patients with non-urothelial histology and 2 patients without data of gender	3 patients without survival data, 4 patients without RNA-seq data,10 patients receiving neoadjuvant chemotherapy, and 4 patients diagnosed with NMIBC.	74 patients without available genomic data	26 patients without available genomic and clinical data, 25 patients with non urothelial histology, and 1 patient without data of gender, and 43 patients did not receive immunotherapy	199 patients diagnosed with urothelial carcinoma
Data analysis	101 patients included in the clincopathological, radiological analysis with targeted sequencing data.	132 patients included for IHC assay and survival analysis.	1218 patients included in survival and genomic analysis.	391 patients included in survival, genomic and transcriptomic analysis.	274 patients included for evaluation of response to checkpoint blockade and genomic features.	123 patients included for evaluation of genomic alteration and clinical features, with 109 patients available with adequate survival data.	199 patients included for survival analysis and genomic alteration.

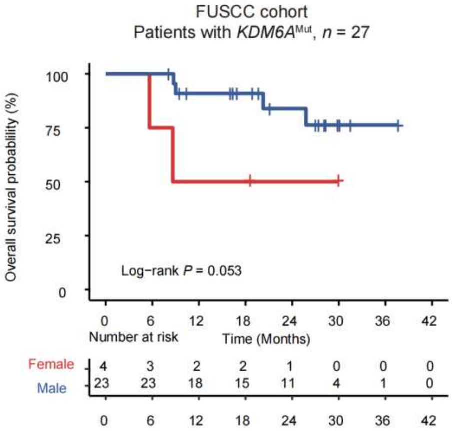
Supplementary Figure 1. Enrollment, clinicopathological features and inclusion criteria for FUSCC, ZSHS, TCGA, MSKCC, IMvigor210, UC-GENOME and MSKCC-IMPACT cohorts.



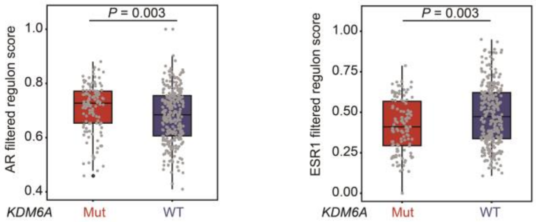
Supplementary Figure 2. Mutational landscape of *KDM6A* in UC. (A) Lollipop plot demonstrating the mutation loci of *KDM6A* in the FUSCC, TCGA, MSKCC, UC-GENOME and MSKCC-IMPACT cohorts. **(B)** Bar plot demonstrating the sexual distribution of *KDM6A* mutation status.



Supplementary Figure 3. Immunohistochemistry characterization of *KDM6A*$^{\mathrm{Mut}}$ samples. (A) Boxplot demonstrating the difference in transcriptomic expression of *KDM6A* in *KDM6A*$^{\mathrm{Mut}}$ and *KDM6A*$^{\mathrm{WT}}$ samples. **(B)** Representative images of immunohistochemistry of KDM6A in the tissue of urothelial bladder cancer.



Supplementary Figure 4. *KDM6A*$^{\mathbf{Mut}}$ male patients are associated with better outcome in the FUSCC cohort. Kaplan-Meier analyses of overall survival between male and female patients with *KDM6A* mutations of FUSCC cohort.



Supplementary Figure 5. AR and ESR1 activity in *KDM6A*$^{\mathrm{Mut}}$ and *KDM6A*$^{\mathrm{WT}}$ patients. Boxplot demonstrating the difference of AR and ESR1 filtered regulon activity in *KDM6A*$^{\mathrm{Mut}}$ and *KDM6A*$^{\mathrm{WT}}$ patients.

Supplementary Table 1. Clinical and Demographic Characteristics of the Patients at Baseline of Public Data Sets.

Cohort	MSKCC cohort (*n* = 1218)		TCGA cohort (*n* = 391)		IMvigor210 cohort (*n* = 274)		UC-GENOME cohort (*n* = 123)		MSKCC-IMPACT cohort (*n* = 199)	
Gender	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	928	290	285	106	218	56	98	25	157	42
Age										
Median age (range) – yr.	66 (18-92)	67 (25-88)	68 (34-90)	73 (43-90)	NA		67 (28-85)	72 (53-83)	68 (32-89)	70 (43-83)
Distribution - no. (%)										
<65	373 (40.2%)	103 (35.5%)	108 (37.9%)	37 (34.9%)			41 (41.8%)	6 (24.0%)	61 (38.9%)	17 (40.5%)
≥65	463 (49.9%)	148 (51.0%)	177 (62.1%)	69 (65.1%)			57 (58.2%)	19 (76.0%)	96 (61.1%)	25 (59.5%)
NA	92 (9.9%)	39 (13.5%)	0	0			0	0	0	0
Median OS (range) – mo.	90.2 (0-104.9)	Not Reached (0-103.6)	35.0 (0.4-165.9)	31.2 (0.6-163.2)	9.6 (0.2-24.5)	9.3 (0.4-23.5)	56.1 (5.3-209.2)	37.8 (3.8-88.3)	16.0 (0-43.0)	15.0 (0-26.0)
ECOG Performance Status - no. (%)										
0	NA		24 (8.4%)	6 (5.7%)	84 (38.5%)	22 (39.3%)	41 (41.8%)	9 (36.0%)	NA	
1			35 (12.3%)	17 (16.0%)	120 (55.1%)	31 (55.4%)	41 (41.8%)	10 (40.0%)		
≥2			10 (3.5%)	4 (3.8%)	14 (6.4%)	3 (5.3%)	16 (16.4%)	6 (24.0%)		
NA			216 (75.8%)	79 (74.5%)	0	0	0	0		
***KDM6A* Mutation Status - no. (%)**										
KDM6A^{Mut}	297 (32.0%)	113 (39.0%)	90 (31.6%)	27 (25.5%)	41 (18.8%)	9 (16.1%)	23 (23.5%)	7 (28.0%)	31 (19.7%)	14 (33.3%)
KDM6A^{WT}	631 (68.0%)	177 (61.0%)	195 (68.4%)	79 (74.5%)	177 (81.2%)	47 (83.9%)	75 (76.5%)	18 (72.0%)	126 (80.3%)	28 (66.7%)

Abbreviations: ECOG: Eastern Cooperative Oncology Group; Mut, mutation; WT, wild type; NA, not available.

Supplementary Table 2. Sources and Details of Gene Signature.

Signature	**Source**
AR filtered regulon signature	PMID: 36273937
ESR1 filtered regulon signature	PMID: 36273937
Tertiary lymphoid structure signature	PMID: 23097687
Angiogenesis signature	PMID: 36494005