



Araştırma Makalesi/Research Article

KMT2D Mutation in Bladder Urothelial Carcinoma: Tumor Mutational Burden, Epigenetic Co-Mutation Profile, and Methylation-Expression Associations

Mesane Ürotelyal Karsinomunda KMT2D Mutasyonu: Tümör Mutasyon Yüğü, Epigenetik Eş Mutasyon Profili ve Metilasyon-Ekspresyon İlişkileri

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Öz

Amaç: Bu çalışmada The Cancer Genome Atlas mesane kanseri kohortunda KMT2D-mutant mesane ürotelyal karsinomlarının klinikopatolojik özellikler, tümör mutasyon yükü, epigenetik düzenleyici gen eş mutasyonları ve KMT2D metilasyon-ekspresyon ilişkileri birlikte değerlendirilerek moleküler profilinin tanımlanması amaçlandı.

Materyal-Metod: Somatik mutasyon verisi bulunan 410 primer mesane ürotelyal karsinomu örneği cBioPortal üzerinden analiz edildi. En az bir protein değiştirici KMT2D mutasyonu taşıyan tümörler KMT2D-mutant olarak sınıflandırıldı. Klinikopatolojik değişkenler, tümör mutasyon yükü, seçilmiş kromatin düzenleyici gen mutasyonları ve KMT2D metilasyon-ekspresyon ilişkileri incelendi. Eş mutasyon sıklıkları Fisher exact testi ile karşılaştırıldı ve Benjamini-Hochberg düzeltmesi uygulandı. KMT2D metilasyon-ekspresyon ve kopya sayı-ekspresyon ilişkileri MEXPRESS verileriyle değerlendirildi.

Bulgular: KMT2D mutasyonu 410 tümörün 115'inde (%28.0) saptandı. KMT2D-mutant tümörlerde tümör mutasyon yükü KMT2D-wild-type tümörlere göre belirgin olarak daha yüksekti; medyan değerler sırasıyla 7.73 ve 5.27 mutasyon/megabaz idi. KMT2C ve NCOR1 mutasyonları KMT2D-mutant tümörlerde false discovery rate düzeltmesi sonrası anlamlı olarak zenginleşti. KMT2C mutasyonu KMT2D-mutant tümörlerin %34.8'inde, wild-type tümörlerin %13.6'sında; NCOR1 mutasyonu ise sırasıyla %14.8 ve %5.4 oranında saptandı. Metilasyon-ekspresyon analizinde KMT2D ekspresyonu ile ters ilişkili dokuz CpG probu belirlendi ve KMT2D kopya sayı değişimi ekspresyon ile pozitif korelasyon gösterdi.

Sonuç: KMT2D-mutant mesane ürotelyal karsinomları artmış mutasyon yükü, seçilmiş epigenetik eş mutasyonların zenginleşmesi ve ölçülebilir metilasyon-ekspresyon ilişkileri ile karakterize moleküler bir alt grup görünümündedir. Bulgular, KMT2D değişikliğinin mesane kanserinin genomik ve epigenetik yapısında daha geniş bir rolü olabileceğini desteklemektedir.

Anahtar Kelimeler: KMT2D, Mesane ürotelyal karsinomu, Tümör mutasyon yükü, DNA metilasyonu, Epigenetik düzenleme, cBioPortal, MEXPRESS

Abstract

Objective: This study aimed to define the molecular profile of KMT2D-mutant bladder urothelial carcinoma by integrating clinicopathological variables, tumor mutational burden, epigenetic regulator gene co-mutations, and KMT2D methylation-expression relationships in The Cancer Genome Atlas bladder cancer cohort.

Materials-Methods: A total of 410 primary bladder urothelial carcinoma samples with available somatic mutation data were analyzed through cBioPortal. Tumors carrying at least one protein-altering KMT2D mutation were classified as KMT2D-mutant. Clinicopathological features, tumor mutational burden, selected chromatin-regulatory gene mutations, and KMT2D methylation-expression associations were evaluated. Co-mutation frequencies were compared using Fisher's exact test with Benjamini-Hochberg correction. KMT2D methylation-expression and copy-number-expression relationships were assessed using MEXPRESS-derived data.

Results: KMT2D mutations were detected in 115 of 410 tumors (28.0%). KMT2D-mutant tumors showed a significantly higher tumor mutational burden than KMT2D-wild-type tumors, with median values of 7.73 and 5.27 mutations per megabase, respectively. KMT2C and NCOR1 mutations were significantly enriched in KMT2D-mutant tumors after false discovery rate correction. KMT2C mutations were detected in 34.8% of KMT2D-mutant tumors compared with 13.6% of wild-type tumors, while NCOR1 mutations were detected in 14.8% and 5.4%, respectively. Methylation-expression analysis identified nine CpG probes inversely correlated with KMT2D expression, and KMT2D copy-number variation showed a positive correlation with expression.

Conclusions: KMT2D-mutant bladder urothelial carcinoma appears to represent a molecular subgroup characterized by increased mutation burden, enrichment of selected epigenetic co-mutations, and measurable methylation-expression associations. These findings support a broader role for KMT2D alteration in the genomic and epigenetic architecture of bladder cancer.

Keywords: KMT2D, Bladder urothelial carcinoma, Tumor mutational burden, DNA methylation, Epigenetic regulation, cBioPortal, MEXPRESS

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INTRODUCTION

Bladder urothelial carcinoma is a clinically and molecularly heterogeneous disease. Tumors with similar histopathological appearance may differ in mutation burden, copy-number alterations, transcriptional programs, molecular subtype, and pathway activity. The first TCGA analysis of urothelial bladder carcinoma and the later expanded TCGA analysis of muscle-invasive bladder cancer showed frequent alterations in cell-cycle regulation, DNA damage response, receptor tyrosine kinase signaling, chromatin regulation, and transcriptional control^{1,2}. These molecular data also supported the development of basal, luminal, and related transcriptomic classifications of muscle-invasive bladder cancer³⁻⁵. Therefore, bladder urothelial carcinoma should be evaluated not only as a histological diagnosis, but also as a disease shaped by distinct genomic and epigenomic backgrounds.

Chromatin-regulatory gene alterations are among the most consistent molecular findings in bladder cancer. Exome sequencing and integrated genomic studies have shown that genes involved in histone modification, chromatin remodeling, enhancer regulation, and transcriptional control are frequently mutated in urothelial tumors^{1,2,6}. Alterations affecting KDM6A, ARID1A, KMT2D, KMT2C, EP300, CREBBP, and STAG2 have been repeatedly reported in bladder cancer cohorts⁶⁻⁸. These findings suggest that disruption of chromatin regulation is not a rare

secondary event, but one of the common molecular features of bladder cancer biology. KMT2D encodes a large histone lysine methyltransferase, also known as MLL4, that contributes to mono- and dimethylation of histone H3 lysine 4 at enhancer regions. Experimental work has shown that MLL4/KMT2D is required for enhancer activation during cell differentiation⁹. KMT2D has also been linked to cell-type-specific gene expression, differentiation, and tumor-suppressive transcriptional programs¹⁰. Its activity and stability are connected to enhancer-associated H3K4 methylation, and disruption of KMT2D may disturb enhancer-driven transcriptional regulation¹¹. Since enhancers control many lineage-specific and tumor-suppressive genes, KMT2D alteration may have broad transcriptional consequences. In bladder cancer, KMT2D has already been studied as a recurrently altered chromatin modifier. Functional work has suggested that KMT2D may inhibit bladder cancer cell growth and metastasis by maintaining tumor suppressor gene expression¹². A previous TCGA-based study by Ding et al. evaluated KMT2D mutation distribution, copy-number variation, messenger RNA expression, microRNA expression, clinical outcome, and pathway-level associations in bladder cancer¹³. More recently, experimental work in urothelial models showed that loss of Kmt2c or Kmt2d can impair urothelial differentiation and sensitize the urothelium to oncogenic transformation¹⁴. These studies support the

biological relevance of KMT2D in urothelial tumorigenesis, but they also indicate that KMT2D should be interpreted within a broader chromatin-regulatory context.

KMT2D mutation may not act as an isolated genomic event. KMT2D functions together with other enhancer-associated regulators, including KMT2C and related transcriptional regulatory complexes^{9,10}. In large-scale cancer genomics, co-occurrence and mutual exclusivity patterns are informative because tumors may accumulate alterations in genes affecting related pathways or compensatory regulatory systems¹⁵. In bladder cancer, co-alterations involving chromatin regulators may therefore help define molecular subgroups with wider disruption of epigenetic regulation. For this reason, comparing KMT2D-mutant and KMT2D-wild-type tumors for selected epigenetic regulator gene mutations may add information beyond the frequency of KMT2D mutation alone.

Tumor mutational burden is another important molecular feature in bladder cancer. Somatic mutation burden is influenced by background mutational processes, DNA damage, repair mechanisms, and tumor evolutionary history. However, interpretation of TMB requires caution because large genes may be more likely to acquire mutations in tumors with high background mutation rates¹⁶. TMB has also been studied as a biomarker for immune checkpoint blockade in several cancers, including urothelial carcinoma, but its clinical interpretation requires treatment-specific outcome data¹⁷. Experimental work has linked

KMT2D deficiency with increased tumor immunogenicity and immune checkpoint blockade sensitivity in model systems¹⁸. Nevertheless, in retrospective public datasets without uniform treatment information, higher TMB should be reported as a genomic feature rather than direct evidence of immunotherapy response.

Molecular profiling of non-muscle-invasive and muscle-invasive bladder cancer has also shown that mutation burden and chromatin-regulatory alterations vary across disease stage and molecular context¹⁹. This supports the need to interpret KMT2D mutation together with the genomic background of the tumor. DNA methylation and copy-number variation may also influence KMT2D transcript abundance. MEXPRESS allows TCGA-based evaluation of gene expression, DNA methylation, copy-number variation, and clinical annotations in the same framework²⁰. Evaluating methylation-expression relationships across the KMT2D locus may therefore complement mutation-based analysis.

The present study aimed to characterize KMT2D-mutant bladder urothelial carcinoma using publicly available TCGA-BLCA data. KMT2D-mutant and KMT2D-wild-type tumors were compared according to clinicopathological characteristics, tumor mutational burden, selected epigenetic regulator gene mutations, and KMT2D methylation-expression relationships. The main aim was to determine whether KMT2D mutation is associated with a distinct genomic

and epigenetic profile in bladder urothelial carcinoma.

MATERIALS AND METHODS

Data source and cohort selection

Clinical and molecular data were obtained from the Bladder Urothelial Carcinoma cohort of The Cancer Genome Atlas PanCancer Atlas through cBioPortal for Cancer Genomics^{21,22}. This was a retrospective bioinformatics cohort study based on publicly available and de-identified data. No new patient recruitment, tissue sampling, or laboratory experiment was performed.

Only primary bladder urothelial carcinoma samples with available somatic mutation data were included in the mutation-based analyses. The TCGA-BLCA dataset contained 411 primary tumor samples. One sample without available somatic mutation profiling was excluded. The final analytical cohort included 410 primary tumor samples.

The clinical variables used in the study were age at diagnosis, sex, sample type, tumor type, AJCC pathological stage, histological grade, pathological T category, pathological N category, pathological M category, overall survival status, recorded survival time, and tumor mutational burden.

Definition of KMT2D mutation status

KMT2D somatic mutation data were downloaded from cBioPortal. Tumors with at least one protein-altering KMT2D mutation were classified as KMT2D-mutant. Tumors with available somatic mutation profiling but

without a KMT2D mutation were classified as KMT2D-wild-type. Missense mutations, nonsense mutations, frameshift deletions, frameshift insertions, splice-site mutations, and in-frame deletions were included in the KMT2D-mutant group. If more than one KMT2D mutation was detected in the same tumor, that tumor was counted once for patient-level group comparisons.

KMT2D mutation events were also summarized separately to describe the mutation spectrum. Mutation-event counts were used only for descriptive mutation-spectrum analysis and were not used to change the patient-level KMT2D-mutant or KMT2D-wild-type classification.

Clinicopathological comparison and tumor mutational burden analysis

Clinicopathological features were compared between KMT2D-mutant and KMT2D-wild-type tumors. Age at diagnosis and recorded survival time were analyzed as continuous variables. Sex, AJCC pathological stage, histological grade, pathological T category, pathological N category, pathological M category, and overall survival status were analyzed as categorical variables.

Tumor mutational burden was obtained from the TCGA-BLCA PanCancer Atlas clinical data as nonsynonymous tumor mutational burden. TMB values were compared between the KMT2D-mutant and KMT2D-wild-type groups. Because TMB values were not normally distributed, TMB was reported as median with interquartile range. Mean and

standard deviation were also given for descriptive purposes. The Mann–Whitney U test was used for the comparison of TMB between the two groups.

Epigenetic co-mutation analysis

A selected panel of chromatin-regulatory and epigenetic regulator genes was evaluated according to KMT2D mutation status. The panel included KMT2C, KDM6A, ARID1A, ARID2, CREBBP, EP300, SETD2, KMT2A, KMT2B, CHD4, STAG2, ASXL1, NCOR1, and EZH2.

Somatic mutation data for these genes were downloaded from cBioPortal. Fusion events were excluded from the mutation counts. For each gene, mutation frequency was calculated separately in KMT2D-mutant and KMT2D-wild-type tumors.

Mutation frequencies were compared using Fisher's exact test. Odds ratios were calculated to show the direction and magnitude of association. The Benjamini–Hochberg method was used to control the false discovery rate across the epigenetic regulator gene panel²³.

KMT2D methylation-expression analysis

DNA methylation and expression data across the KMT2D locus were obtained from MEXPRESS-derived TCGA-BLCA data²⁰. Available CpG methylation probes in the KMT2D gene region were evaluated together with KMT2D expression values. For each CpG probe with valid paired methylation and

expression data, methylation beta values were correlated with KMT2D expression using Pearson correlation analysis. The Benjamini–Hochberg method was applied for multiple testing correction across CpG-level comparisons²³. CpG probes with an FDR value below 0.05 were considered statistically significant.

KMT2D copy-number variation was also evaluated in relation to KMT2D expression, because gene dosage may affect transcript abundance. The CNV-expression correlation was analyzed separately and was not included in the CpG-level FDR correction.

Statistical analysis

Continuous variables were summarized as mean $\pm$ standard deviation and/or median with interquartile range. Categorical variables were summarized as number and percentage. The Mann–Whitney U test was used for continuous variables. The chi-square test or Fisher's exact test was used for categorical variables, according to expected cell counts.

For the epigenetic co-mutation analysis, Fisher's exact test was used for each gene, and odds ratios were reported. False discovery rate correction was performed using the Benjamini–Hochberg method. For methylation-expression analysis, Pearson correlation coefficients and p values were calculated, followed by Benjamini–Hochberg correction for CpG-level comparisons. A two-sided p value below 0.05 was accepted as statistically significant unless an FDR-adjusted value was reported.

RESULTS

Cohort characteristics

A total of 410 primary TCGA-BLCA tumors with available somatic mutation data were included in the final analysis. The cohort consisted of 303 male patients (73.9%) and 107 female patients (26.1%). The mean age at diagnosis was 68.11 years, with a range of 34 to 90 years. All included samples were primary tumor samples.

Overall survival status was recorded as alive in 229 patients (55.9%) and deceased in 181 patients (44.1%). The median recorded survival time was 17.62 months. Most tumors were classified as muscle-invasive urothelial carcinoma (pT2 or above) (407/410, 99.3%). According to AJCC pathological stage, 2 tumors were stage I (0.5%), 130 were stage II (31.7%), 140 were stage III (34.1%), and 136 were stage IV (33.2%). Histological grade was high grade in 386 tumors (94.1%) and low grade in 21 tumors (5.1%). The median nonsynonymous tumor mutational burden of the cohort was 5.77 mut/Mb. The clinicopathological characteristics of the cohort are shown in Table 1.

KMT2D mutation frequency and mutation spectrum

KMT2D mutation status was evaluated in 410 primary TCGA-BLCA tumors with available

somatic mutation data. KMT2D mutations were detected in 115 tumors (28.0%), whereas 295 tumors (72.0%) were classified as KMT2D-wild-type.

Table 1. Clinicopathological characteristics of the TCGA-BLCA cohort

Characteristic	Value
Sex	
Male	303 (73.9)
Female	107 (26.1)
Age at diagnosis, years	68.11 (34–90)
Sample type	
Primary tumor	410 (100.0)
Overall survival status	
Alive	229 (55.9)
Deceased	181 (44.1)
Recorded survival time, months	Median: 17.62
Cancer type	
Bladder urothelial carcinoma	410 (100.0)
Tumor type	
Muscle invasive urothelial carcinoma (pT2 or above)	407 (99.3)
Bladder urothelial carcinoma	3 (0.7)
cBioPortal subtype	
BLCA	399 (97.3)
Not available	11 (2.7)
AJCC pathological stage	
Stage I	2 (0.5)
Stage II	130 (31.7)
Stage III	140 (34.1)
Stage IV	136 (33.2)
Not available	2 (0.5)
Histological grade	
High grade	386 (94.1)
Low grade	21 (5.1)
Not available	3 (0.7)
TMB, nonsynonymous mutations/Mb	Median: 5.77

A total of 160 KMT2D mutation events were identified among the 115 KMT2D-mutant tumors. The most frequent mutation types were nonsense mutations (60/160, 37.5%) and missense mutations (58/160, 36.3%). These were followed by frameshift deletions (20/160, 12.5%), splice-site mutations (12/160, 7.5%), frameshift insertions (9/160, 5.6%), and in-frame deletions (1/160, 0.6%). Overall, truncating or splice-site alterations accounted for 101 of 160 mutation events (63.1%). Multiple KMT2D mutations were observed in 36 tumors.

Clinicopathological comparison according to KMT2D mutation status

Clinicopathological features were compared between KMT2D-mutant and KMT2D-wild-type tumors. No statistically significant difference was observed between the two groups in age at diagnosis, sex, AJCC

pathological stage, histological grade, pathological T category, pathological N category, pathological M category, overall survival status, or recorded survival time.

The mean age at diagnosis was 69.08 ± 11.07 years in the KMT2D-mutant group and 67.74 ± 10.40 years in the KMT2D-wild-type group ($p = 0.220$). Male sex was present in 88 KMT2D-mutant cases (76.5%) and 215 KMT2D-wild-type cases (72.9%) ($p = 0.532$). Overall survival status also did not differ significantly between groups; 70 patients in the KMT2D-mutant group (60.9%) and 159 patients in the KMT2D-wild-type group (53.9%) were alive at the recorded follow-up point ($p = 0.224$). Median recorded survival time was 18.90 months in the KMT2D-mutant group and 16.47 months in the KMT2D-wild-type group ($p = 0.277$). The full comparison of clinicopathological variables is shown in Table 2.

Table 2. Comparison of clinicopathological features and tumor mutational burden between KMT2D-mutant and KMT2D-wild-type bladder cancer cases

Characteristic	KMT2D-mutant (n=115)	KMT2D-wild-type (n=295)	p value
KMT2D status	115 (28.0)	295 (72.0)	
Age at diagnosis, years, mean $\pm$ SD	69.08 $\pm$ 11.07	67.74 $\pm$ 10.40	0.220
Age at diagnosis, years, median (IQR)	71.00 (60.50-77.50)	68.00 (60.00-76.00)	0.220
Sex	Male: 88 (76.5); Female: 27 (23.5)	Male: 215 (72.9); Female: 80 (27.1)	0.532
AJCC pathological stage	Stage I: 0 (0.0); Stage II: 40 (34.8); Stage III: 44 (38.3); Stage IV: 31 (27.0); Not available: 0 (0.0)	Stage I: 2 (0.7); Stage II: 90 (30.5); Stage III: 96 (32.5); Stage IV: 105 (35.6); Not available: 2 (0.7)	0.321
Histological grade	High grade: 111 (96.5); Low grade: 3 (2.6); Not available: 1 (0.9)	High grade: 275 (93.2); Low grade: 18 (6.1); Not available: 2 (0.7)	0.348
Pathologic T category	T0-T1: 0 (0.0); T2: 33 (28.7); T3: 56 (48.7); T4: 13 (11.3); Not available: 13 (11.3)	T0-T1: 4 (1.4); T2: 86 (29.2); T3: 139 (47.1); T4: 46 (15.6); Not available: 20 (6.8)	0.312
Pathologic N category	N0: 74 (64.3); N1: 10 (8.7); N2: 18 (15.7); N3: 0 (0.0); Not available: 13 (11.3)	N0: 163 (55.3); N1: 37 (12.5); N2: 58 (19.7); N3: 8 (2.7); Not available: 29 (9.8)	0.180
Pathologic M category	M0: 53 (46.1); M1: 5 (4.3); Not available: 57 (49.6)	M0: 142 (48.1); M1: 6 (2.0); Not available: 147 (49.8)	0.422
Overall survival status	Alive: 70 (60.9); Deceased: 45 (39.1)	Alive: 159 (53.9); Deceased: 136 (46.1)	0.224
Overall survival time, months, median (IQR)	18.90 (11.97-30.74)	16.47 (10.79-32.76)	0.277
Tumor mutational burden, mut/Mb, mean $\pm$ SD	12.85 $\pm$ 15.76	6.72 $\pm$ 5.65	4.66 $\times 10^{-7}$
Tumor mutational burden, mut/Mb, median (IQR)	7.73 (4.72-14.28)	5.27 (2.85-8.83)	4.66 $\times 10^{-7}$

Note: Values are presented as n (%) unless otherwise stated. Continuous variables are shown as mean $\pm$ SD or median (IQR). P values were calculated using the Mann-Whitney U test for continuous variables and chi-square or Fisher's exact test for categorical variables, as appropriate. KMT2D-mutant tumors were defined by the presence of at least one protein-altering KMT2D mutation. TMB, tumor mutational burden; IQR, interquartile range; SD, standard deviation.

Tumor mutational burden according to KMT2D mutation status

Tumor mutational burden differed significantly according to KMT2D mutation status. KMT2D-mutant tumors had higher TMB values than KMT2D-wild-type tumors. The median TMB was 7.73 mut/Mb in the KMT2D-mutant group and 5.27 mut/Mb in the KMT2D-wild-type group. Mean TMB was also higher in KMT2D-mutant tumors than in KMT2D-wild-type tumors (12.85 ± 15.76 vs. 6.72 ± 5.65 mut/Mb). The difference in TMB between groups was statistically significant by Mann–Whitney U test ($p = 4.66 \times 10^{-7}$). This finding is shown in Table 2 and Figure 1.

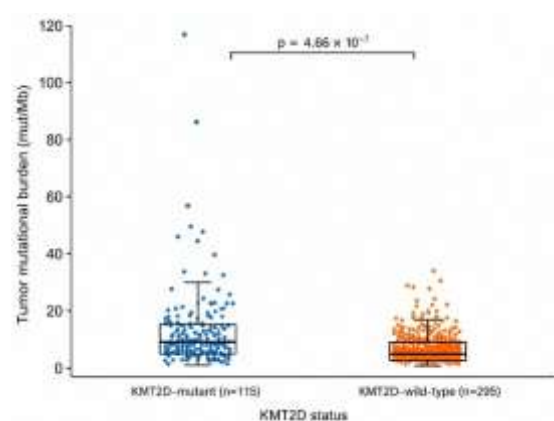


Figure 1. Tumor mutational burden according to KMT2D mutation status in TCGA-BLCA. Tumor mutational burden was significantly higher in KMT2D-mutant tumors than in KMT2D-wild-type tumors. Boxes indicate the interquartile range, horizontal lines indicate the median, and whiskers show the distribution of values. Each dot represents one primary tumor sample. The difference between groups was assessed using the Mann–Whitney U test ($p = 4.66 \times 10^{-7}$).

Epigenetic co-mutation profile

Epigenetic regulator gene mutations were compared between KMT2D-mutant and KMT2D-wild-type tumors. After exclusion of fusion events and false discovery rate correction, KMT2C and NCOR1 mutations remained significantly enriched in the KMT2D-mutant group.

KMT2C mutations were detected in 40 of 115 KMT2D-mutant tumors (34.8%) and in 40 of 295 KMT2D-wild-type tumors (13.6%). This difference was statistically significant (odds ratio = 3.40, $p = 3.19 \times 10^{-6}$, FDR = 4.47×10^{-5}). NCOR1 mutations were also more frequent in KMT2D-mutant tumors than in KMT2D-wild-type tumors. NCOR1 mutations were present in 17 of 115 KMT2D-mutant tumors (14.8%) and in 16 of 295 KMT2D-wild-type tumors (5.4%) (odds ratio = 3.02, $p = 0.0038$, FDR = 0.0267). CREBBP, EP300, and ASXL1 showed higher nominal mutation frequencies in KMT2D-mutant tumors, but these differences did not remain statistically significant after false discovery rate correction. Other evaluated epigenetic regulator genes did not show significant differences between the two groups. The epigenetic co-mutation results are presented in Table 3. The co-alteration pattern of KMT2D, KMT2C, and NCOR1 is shown in Figure 2.

Table 3. Epigenetic regulator gene mutations according to KMT2D mutation status in TCGA-BLCA

Gene	KMT2D-mutant (%)	n/N	KMT2D-wild-type (%)	n/N	Odds ratio	p value	FDR
KMT2C	40/115 (34.8)		40/295 (13.6)		3.40	3.19×10^{-6}	4.47×10^{-5}
KDM6A	22/115 (19.1)		83/295 (28.1)		0.60	0.077	0.181
ARID1A	28/115 (24.3)		77/295 (26.1)		0.91	0.801	0.885
ARID2	9/115 (7.8)		23/295 (7.8)		1.00	1.000	1.000
CREBBP	20/115 (17.4)		29/295 (9.8)		1.93	0.042	0.181
EP300	24/115 (20.9)		39/295 (13.2)		1.73	0.067	0.181
SETD2	8/115 (7.0)		18/295 (6.1)		1.15	0.822	0.885
KMT2A	17/115 (14.8)		28/295 (9.5)		1.65	0.158	0.277
KMT2B	9/115 (7.8)		12/295 (4.1)		2.00	0.137	0.273
CHD4	9/115 (7.8)		14/295 (4.7)		1.70	0.236	0.368
STAG2	18/115 (15.7)		38/295 (12.9)		1.26	0.522	0.664
ASXL1	11/115 (9.6)		13/295 (4.4)		2.29	0.060	0.181
NCOR1	17/115 (14.8)		16/295 (5.4)		3.02	0.0038	0.0267
EZH2	3/115 (2.6)		3/295 (1.0)		2.61	0.356	0.498

Note: Fusion events were excluded from mutation counts. P values were calculated using Fisher's exact test. False discovery rate correction was performed using the Benjamini-Hochberg method. FDR, false discovery rate.

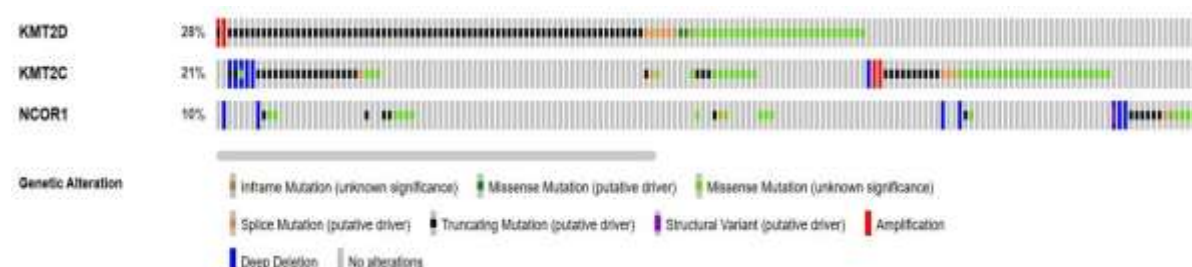


Figure 2. Co-alteration pattern of KMT2D, KMT2C, and NCOR1 in TCGA-BLCA. The OncoPrint shows genomic alterations affecting KMT2D, KMT2C, and NCOR1 in the TCGA-BLCA cohort. Statistical comparisons between KMT2D-mutant and KMT2D-wild-type tumors are presented in Table 3.

KMT2D methylation-expression associations

KMT2D methylation-expression relationships were evaluated using MEXPRESS-derived TCGA-BLCA data. Among the CpG probes available across the KMT2D locus, nine probes

showed significant inverse correlations with KMT2D expression after false discovery rate correction.

The strongest inverse association was observed for cg00522588 (Pearson $r = -0.345$, $p = 7.16 \times$

10^{-13} , $FDR = 2.22 \times 10^{-11}$). Other CpG probes with significant negative correlations included cg09643371, cg21580220, cg08163578, cg19253410, cg02831219, cg08089780, cg11229610, and cg13007988. KMT2D copy-number variation was positively correlated with KMT2D expression (Pearson $r = 0.313$, $p = 1.24 \times 10^{-10}$). The CpG methylation-expression and CNV-expression results are shown in Table 4.

DISCUSSION

In this study, KMT2D-mutant bladder urothelial carcinomas were evaluated using TCGA-BLCA

data. KMT2D mutations were detected in 28.0% of primary tumors with available somatic mutation data. The main finding was that KMT2D-mutant tumors had higher tumor mutational burden than KMT2D-wild-type tumors. KMT2D-mutant tumors also showed enrichment of KMT2C and NCOR1 mutations after false discovery rate correction. Methylation-expression analysis showed inverse correlations between several CpG probes across the KMT2D locus and KMT2D

Table 4. KMT2D methylation-expression associations in TCGA-BLCA

CpG probe	n	Pearson r	p value	FDR
cg00522588	408	-0.345	7.16×10^{-13}	2.22×10^{-11}
cg09643371	408	-0.217	9.52×10^{-6}	1.48×10^{-4}
cg21580220	408	-0.204	3.27×10^{-5}	3.38×10^{-4}
cg08163578	408	-0.191	1.04×10^{-4}	8.07×10^{-4}
cg19253410	408	-0.184	1.94×10^{-4}	1.11×10^{-3}
cg02831219	408	-0.182	2.15×10^{-4}	1.11×10^{-3}
cg08089780	408	-0.176	3.45×10^{-4}	1.53×10^{-3}
cg11229610	407	-0.157	1.46×10^{-3}	5.66×10^{-3}
cg13007988	408	-0.152	2.02×10^{-3}	6.94×10^{-3}
KMT2D CNV	404	0.313	1.24×10^{-10}	—

Note: CpG methylation beta values were correlated with KMT2D expression using Pearson correlation analysis. FDR correction was applied across CpG probes. KMT2D copy-number variation was analyzed separately and was not included in CpG-level FDR correction. CNV, copy-number variation; FDR, false discovery rate.

expression. KMT2D copy-number variation was also positively correlated with KMT2D expression.

The observed KMT2D mutation frequency is consistent with the molecular profile of bladder cancer. TCGA studies showed that bladder tumors frequently carry alterations in genes involved in chromatin regulation, together with changes affecting cell-cycle control, DNA damage response, receptor tyrosine kinase signaling, and transcriptional programs^{1,2}. Earlier sequencing studies reported frequent mutations in chromatin-regulatory genes in urothelial carcinoma⁶.

Additional bladder cancer studies also support the functional and clinicopathological relevance of chromatin-remodeling alterations, including STAG2 and other chromatin regulator genes^{24,25}. Broader cancer epigenomics studies have shown that DNA methylation and chromatin-related mechanisms can influence cancer biology and gene regulation^{26,27}. Background mutational processes differ between tumors and can shape the total number and pattern of somatic mutations²⁸. Pan-cancer molecular analyses further showed that tumor classification is strongly influenced by cell-of-origin and lineage-related transcriptional programs²⁹. Large-scale cancer mutation frequency analyses also support that chromatin-regulatory genes, including KMT2D and related regulators, are recurrently altered across human cancers³⁰. In this context, KMT2D mutation should be viewed as part of the wider chromatin-regulatory background of

bladder cancer rather than as a rare isolated event.

KMT2D has a defined role in enhancer regulation. Experimental work showed that MLL4/KMT2D is required for enhancer activation during cell differentiation⁹. KMT2D biology has also been linked to cell-type-specific transcription and tumor-suppressive gene regulation^{10,11}. In bladder cancer, Sun et al. reported that KMT2D may limit tumor growth and metastasis by maintaining tumor suppressor gene expression¹². The present study did not test KMT2D function experimentally. Still, the high proportion of nonsense, frameshift, and splice-site mutation events suggests that many KMT2D alterations in this cohort may have biological relevance.

The TMB result is one of the strongest findings of this analysis. KMT2D-mutant tumors showed significantly higher TMB than KMT2D-wild-type tumors. This finding should not be overinterpreted. KMT2D is a large gene, and large genes may acquire mutations more often in tumors with high background mutation burden¹⁶. Background mutational processes also differ between tumors and can shape the total number and pattern of somatic mutations²⁸. Therefore, KMT2D mutation may partly mark a genomically unstable tumor background. In this cohort, KMT2D-mutant and KMT2D-wild-type tumors did not differ significantly in age, sex, stage, grade, pathological T/N/M category, overall survival status, or recorded survival time. This supports that the observed

TMB difference was not simply due to these recorded clinicopathological variables. However, the analysis remains observational and cannot prove that KMT2D mutation causes increased TMB.

TMB has clinical interest because it has been evaluated as a biomarker for immune checkpoint blockade in different tumor types¹⁷. Experimental work also identified KMT2D as a modulator of immune checkpoint blockade in model systems¹⁸. These findings are relevant biologically, but they should be applied carefully here. We did not analyze immunotherapy-treated patients, immune-cell infiltration, or treatment response. Therefore, the higher TMB in KMT2D-mutant tumors should be reported as a genomic feature, not as evidence of better response to immunotherapy.

The co-mutation analysis showed that KMT2C and NCOR1 mutations were enriched in KMT2D-mutant tumors after FDR correction. KMT2C is closely related to KMT2D and participates in enhancer-associated chromatin regulation. Earlier enhancer studies showed functional cooperation between MLL3/KMT2C and MLL4/KMT2D during enhancer activation^{9,10}. The higher frequency of KMT2C mutations in KMT2D-mutant tumors suggests that these cases may carry broader disturbance of enhancer-regulatory machinery. This finding supports the idea that KMT2D-mutant bladder cancers may represent a subgroup with wider disruption of

chromatin regulation rather than a single-gene subgroup.

NCOR1 was also more frequently mutated in the KMT2D-mutant group. NCOR1 is a transcriptional corepressor and participates in chromatin-associated regulatory complexes. Its enrichment may indicate additional disruption of transcriptional control in the KMT2D-mutant group. NCOR1 mutations have also been studied in bladder cancer in relation to tumor immune features and immune checkpoint inhibitor outcomes³¹. The present study did not evaluate immune infiltration or treatment response, so this result should be interpreted only as a co-mutation association. It is still useful because it shows that KMT2D-mutant tumors carry additional alterations in transcriptional regulatory genes. CREBBP, EP300, and ASXL1 showed higher nominal mutation frequencies in KMT2D-mutant tumors, but these associations did not remain significant after FDR correction. This point should be clear. These genes may suggest a tendency toward wider chromatin-regulator involvement, but they are not main positive findings in this study. After multiple-testing correction, only KMT2C and NCOR1 remained significant. Similar caution is needed in interpreting other chromatin-regulator genes, because bladder cancer contains several overlapping epigenetic and chromatin-remodeling alterations^{8,24,25}.

The present findings should also be considered in relation to the previous TCGA-based study by Ding et al.¹³. That study examined KMT2D mutation distribution

together with copy-number variation, mRNA expression, microRNA expression, clinical outcome, and pathway-level associations in bladder cancer. The present study was not designed to repeat the same analysis. Here, KMT2D mutation status was used as the grouping variable, and the analysis focused on TMB, selected epigenetic co-mutations, and methylation-expression patterns in the same cohort. This is the main difference between the two studies.

The methylation-expression analysis showed that nine CpG probes across the KMT2D locus had significant inverse correlations with KMT2D expression after FDR correction. The strongest association was observed for cg00522588. These findings suggest that methylation at selected CpG sites may be linked to lower KMT2D transcript abundance in BLCA. At the same time, KMT2D copy-number variation was positively correlated with KMT2D expression. This indicates that gene dosage may also contribute to transcript-level differences. Since these findings were derived from MEXPRESS-based TCGA data, they should be considered exploratory platform-based associations²⁰. They do not prove a direct regulatory mechanism. This interpretation is consistent with the broader view that DNA methylation and other epigenetic mechanisms can influence gene expression in cancer^{26,27,32}.

No statistically significant differences were observed between KMT2D-mutant and KMT2D-wild-type tumors for the clinicopathological variables evaluated in this

study. Recorded survival time and overall survival status were also not significantly different between the two groups. Since Kaplan-Meier survival modeling was not performed, these findings should be interpreted only as clinicopathological comparisons based on available recorded variables. The study does not claim that KMT2D mutation is a prognostic marker in bladder cancer.

This study has several limitations. First, it was based on retrospective public data and did not include independent experimental validation. Second, treatment details, immunotherapy exposure, and longitudinal clinical data were not uniformly available for all cases. Third, methylation findings were derived from platform-based CpG-level data and should be interpreted as exploratory. Fourth, the co-mutation analysis was limited to a selected epigenetic regulator gene panel. Finally, although KMT2D-mutant tumors showed higher TMB, this association cannot be translated into immunotherapy response or clinical decision-making without dedicated clinical outcome data.

CONCLUSION

In conclusion, KMT2D mutation in The Cancer Genome Atlas bladder urothelial carcinoma cohort was associated with higher tumor mutational burden and enrichment of KMT2C and NCOR1 mutations. KMT2D methylation-expression analysis also showed inverse correlations between several CpG probes and KMT2D expression, together with

a positive relationship between copy-number variation and expression. These findings suggest that KMT2D-mutant bladder urothelial carcinomas may represent a subgroup with increased mutation burden and broader disturbance of epigenetic regulation. Independent cohorts and experimental studies are needed to clarify the biological and clinical significance of these associations. Future studies should also evaluate whether these molecular associations have any relationship with treatment response in clinically annotated bladder cancer cohorts.

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None.

Ethical Approval

This study was based exclusively on publicly available and de-identified data obtained from The Cancer Genome Atlas. No new patient recruitment, tissue sampling, animal experiment, or identifiable patient information was used. Therefore, additional patient informed consent was not required for this secondary bioinformatics analysis.

Conflict of Interest

The author declares no conflict of interest.

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