Integrative Clinical and Genomic Characterization of MTAP-deficient Metastatic Urothelial Cancer

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Abstract

Deficiency of MTAP (MTAP^{def}) mainly occurs because of homozygous loss of chromosome 9p21, which is the most common copy-number loss in metastatic urothelial cancer (mUC). We characterized the clinical and genomic features of MTAP^{def} mUC in 193 patients treated at MD Anderson Cancer Center (MDACC) and 298 patients from the phase 2 IMvigor210 trial, which investigated atezolizumab in cisplatin-ineligible and platinum-refractory disease. In the MDACC cohort, visceral metastases were significantly more common for MTAP^{def} ($n = 48$) than for MTAP-proficient (MTAP^{prof}; $n = 145$) patients (75% vs 55.2%; $p = 0.02$). MTAP^{def} was associated with poor prognosis (median overall survival [mOS] 12.3 vs 20.2 mo; $p = 0.007$) with an adjusted hazard ratio of 1.93 (95% confidence interval 1.35–2.98). Similarly, IMvigor210 patients with $MTAP^{lo}$ ($n = 29$) had a higher incidence of visceral metastases than those with $MTAP^{hi}$ tumors ($n = 269$; 86.2% vs 72.5%; $p = 0.021$) and worse prognosis (mOS 8.0 vs 11.3 mo; $p = 0.042$). Hyperplasia-associated genes were more frequently mutated in MTAP^{def} tumors ($FGFR3$: 31% vs 8%; $PI3KCA$: 31% vs 19%), while alterations in dysplasia-associated genes were less common in MTAP^{def} tumors ($TP53$: 41% vs 67%; $RB1$: 0% vs 16%). Our findings support a distinct biology in MTAP^{def} mUC that is associated with early visceral disease and worse prognosis.

Patient summary:

We investigated the outcomes for patients with the most common gene loss (MTAP gene) in metastatic cancer of the urinary tract. We found that this loss correlates with worse prognosis and a higher risk of metastasis in internal organs. There seems to be distinct tumor biology for urinary tract cancer with MTAP gene loss and this could be a potential target for treatment.

Keywords

Located in chromosomal region 9p21, the *MTAP* gene encodes an enzyme that is essential in the salvage pathway for adenosine and methionine synthesis [1]. Our analysis of focal copy-number losses in The Cancer Genome Atlas muscle-invasive bladder cancer (TCGA-BLCA) cohort ($n = 389$ patients) previously revealed that 27.2% of cancers exhibited homozygous deletion (HD) of *MTAP* that was in all cases associated with concurrent HD of *CDKN2A,* most commonly due to 9p21 loss [2]. We subsequently developed a Clinical Laboratory Improvement Amendments (CLIA)-certified MTAP immunohistochemistry (IHC) test to determine tumor MTAP deficiency (MTAP^{def}) as a clinically accessible surrogate biomarker to investigate the clinical and biological features of urothelial cancer (UC) with 9p21 loss.

We assessed a total of 212 patients who were treated with standard-of-care chemotherapy and immunotherapy at MD Anderson Cancer Center (MDACC; institutional review board protocol PA17–0577) between November 1997 and April 2018 (Supplementary Fig. 1). CLIA-certified IHC staining for MTAP was carried out using a primary anti-MTAP antibody (1:1200, #11475–1-AP; ProteinTech, Rosemont, IL, USA). Cases with any MTAP cytoplasmic staining were defined as MTAP-proficient (MTAP^{prof}), whereas those with complete loss of MTAP staining were defined as MTAP^{def}. Somatic alterations in tumor tissue were detected via targeted sequencing; circulating tumor DNA was utilized if tissue was unavailable [3]. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional-hazards regression models to evaluate the association between patient prognostic variables and median overall survival (mOS). All computations were carried out in SAS v9.4 (SAS Institute, Cary, NC).

A total of 193 patients were included in our study after excluding those with undetermined primary tumor, concurrent metastatic disease, or insufficient tissue for MTAP staining (Supplementary Tables 1–3). Median follow-up was 40.6 mo (33.4–44.6). MTAP^{def} was observed in 24.9% of patients. First-line therapy (Supplementary Table 4) most commonly included chemotherapy (60.6%), followed by immune checkpoint inhibitors (ICIs; 28.5%; Supplementary Fig. 2). Treatment regimens were balanced between the MTAP^{def} and MTAP^{prof} groups (Supplementary Table 4). mOS was significantly shorter for patients with MTAP^{def} UC versus MTAP^{prof} UC (12.3 vs 20.2 mo; $p = 0.007$; Fig. 1A). Similarly, an adverse impact of MTAP^{def} on progression-free survival (PFS) was noted among the 172 patients who received frontline systemic therapy (4.0 vs 5.5 mo; $p = 0.042$; Fig. 1B). Further analysis of the frontline ICI and chemotherapy subgroups demonstrated that mOS and PFS in both subgroups were shorter for patients with MTAP^{def} versus MTAP^{prof} (Supplementary Fig. 3A–D). Since the number of metastatic sites at baseline, baseline visceral metastases, development of subsequent visceral metastases, and Eastern Cooperative Oncology Group performance score 1 were all associated with worse OS (Supplementary Table 5), we conducted a multivariable Cox assessment, which showed an independent adverse impact of MTAP^{def} on OS (HR 1.93, 95% 1.25–2.98; $p = 0.003$; Supplementary Table 5). Furthermore, patients with MTAP^{def} mUC had a higher rate of early visceral metastases (75% vs 55.2%; $p = 0.02$; Fig. 1C, Supplementary Table 6).

In total, 152 patients had target sequencing data available (Fig. 1D). The most frequently mutated genes were *TP53* (59%), *PIK3CA* (20%), *TERT* (18%), *FGFR3* (12%), and *RB1* (12%; Supplementary Table 7, Supplementary Fig. 4). The dysplasia-associated alterations *TP53* and *RB1* were detected more often in MTAP^{prof} tumors versus MTAP^{def} tumors (67% vs 41%; $p < 0.001$; and 16% vs 0%; $p < 0.001$), while the hyperplasia-associated alterations *FGFR3* and *PI3KCA* were more common in MTAP^{def} tumors (31% vs 8%; $p < 0.001$; and 31% vs 19%; $p = 0.05$). The majority of *FGFR2/3* alterations were functional oncogenic mutations (Supplementary Fig. 5).

Prior efforts highlighted two potential pathways involved in the oncogenesis of early-stage UC: the hyperplasia (*FGFR*-driven) pathway in low-grade papillary non–muscle-invasive UC (NMIUC) and the dysplasia (*TP53*- and *Rb1*-driven) pathway in muscle-invasive UC (MIUC) [4,5]. These studies also suggested that low-grade papillary NMIUCs might

progress to MIUCs as a result of *CDKN2A* loss, a well-known tumor-suppressor gene encoding p16 and p14[ARF]. MTAP loss in our bladder cancer cohort is a surrogate for 9p21 loss, most commonly encompassing *CDKN2A*. Our findings of frequent *FGFR* and *PIK3CA* alterations among tumors with MTAP[def] suggest that these metastatic tumors originating from the hyperplasia pathway have distinct genomic drivers, visceral organ involvement, and clinical prognosis as compared to tumors arising from the dysplasia pathway (Supplementary Fig. 6).

We further assessed clinical and bulk RNA sequencing data for cisplatin-ineligible (cohort 1) and platinum-refractory (cohort 2) patients treated with atezolizumab in the IMvigor210 trial [6]. On the basis of *MTAP* transcriptional levels, samples were classified into two groups: the bottom 25% was defined as *MTAP*-low ($MTAP^{lo}$, $n = 75$) and the remainder defined as *MTAP*-high ($MTAP^{hi}$, $n = 223$; Fig. 2A). This 25% threshold was chosen to match the prevalence of MTAP deficiency in the TCGA-BLCA and MDACC cohorts. Patients with $MTAP^{lo}$ versus $MTAP^{hi}$ had a higher risk of visceral metastatic disease (82.6% vs 70.8%; $p = 0.021$; Fig. 2B) and shorter mOS (8.8 vs 12.7 mo; HR 1.4, 95% CI 1.01–1.98; $p = 0.029$; Fig. 2C,D).

The mechanism by which MTAP[def] may be associated with visceral metastases is unclear, but could be related to the hyperplasia pathway by which 9p21 loss promotes superficial tumors to acquire muscle invasion [4,5]. MTAP deficiency results in a buildup of its substrate, methylthioadenosine, which inhibits the methylation of STAT1 and, as a result, inhibits interferon signaling pathways [7]. Defects in these pathways have been associated with compromised antitumor immune responses [8]. Furthermore, loss of cell-cycle control due to concomitant *CDKN2A* HD [9] in this context of immune suppression could explain the early visceral metastases in MTAP[def] mUC. Antifolates may be synthetically lethal in MTAP[def] mUC via targeting of de novo purine synthesis in the presence of defective salvage synthesis of adenine [10]. We are currently investigating the potential synergy between pemetrexed and avelumab (NCT03744793) along with immune-monitoring studies on pre- and post-treatment MTAP[def] tumor tissues.

Our study has the limitations of being a nonrandomized retrospective study from a single institution, with variability in therapy, surveillance, and follow-up times. Furthermore, different methodology (RNA sequencing) was used in the IMvigor210 trial compared to our MDACC cohort (IHC).

In conclusion, our data suggest that MTAP[def] in mUC is associated with worse prognosis and a distinct driving biology that may be targetable. These data are hypothesis-generating and provide a basis for future mechanistic studies for the development of effective combination therapies for MTAP[def] mUC such as antifolates, cell cycle inhibitors, and/or *FGFR* inhibitors. Further studies are required to assess the concordance between 9p21 copy-number alterations and MTAP IHC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

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We investigated the outcomes for patients harboring the most common genomic loss (MTAP loss) in metastatic urothelial cancer. We found that MTAP loss correlates with worse prognosis, a higher risk of visceral involvement, a distinct and potentially targetable tumor biology.
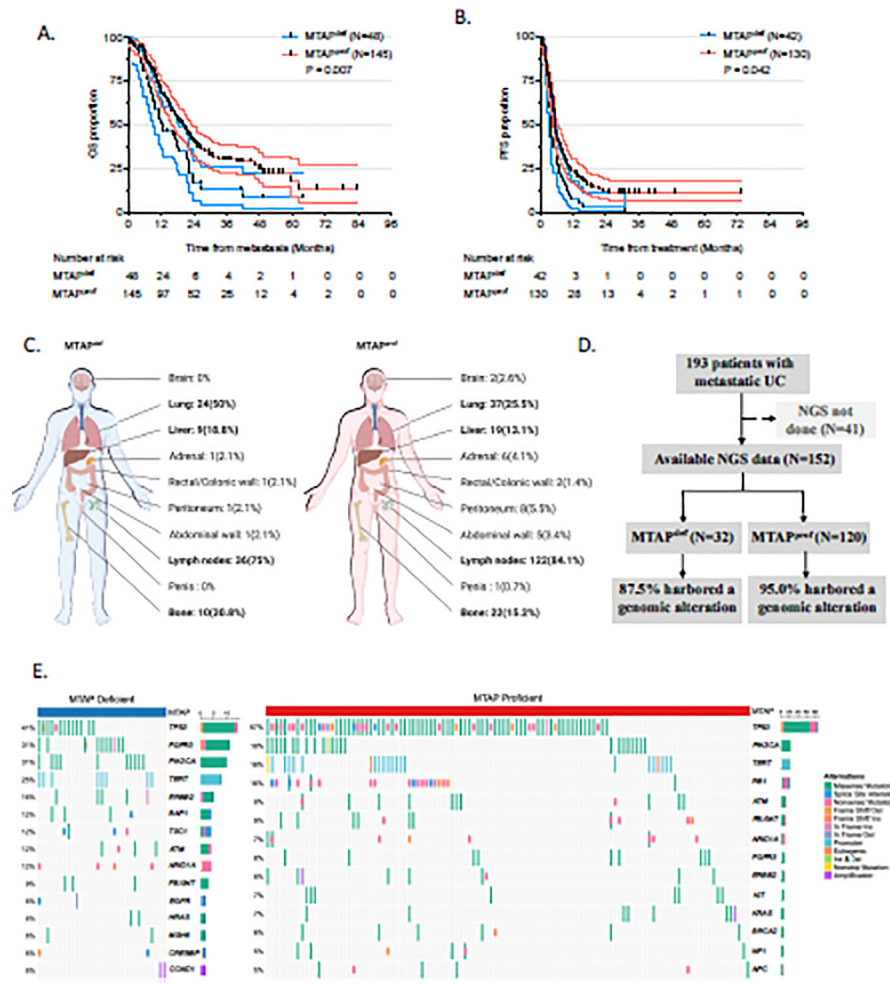
Fig. 1 –.

MTAP^{def} was associated with poor OS and FPS, metastatic status, and genomic alteration. (A) OS for patients stratified by MTAP status. (B) PFS for patients stratified by MTAP status. Only 172 patients who received chemotherapy or immunotherapy as front-line therapy were analyzed for PFS. (C) Proportion of patients by site of metastasis involvement at baseline. Created using a licensed version of biorender.com. (D) CONSORT diagram for patients with available target sequencing data and genomic alterations. (E) Oncoplot showing the most common genomic alterations in our patient cohorts. Patients without genomic alterations and genes with an alteration frequency of <5% in each group were excluded. The right bar plot showed the alteration frequency for each gene. OS = overall survival; PFS = progression-free survival; UC = urothelial cancer; MTAP>^{def} = MTAP-deficient; MTAP^{prof} = MTAP-proficient; NGS = next-generation sequencing.
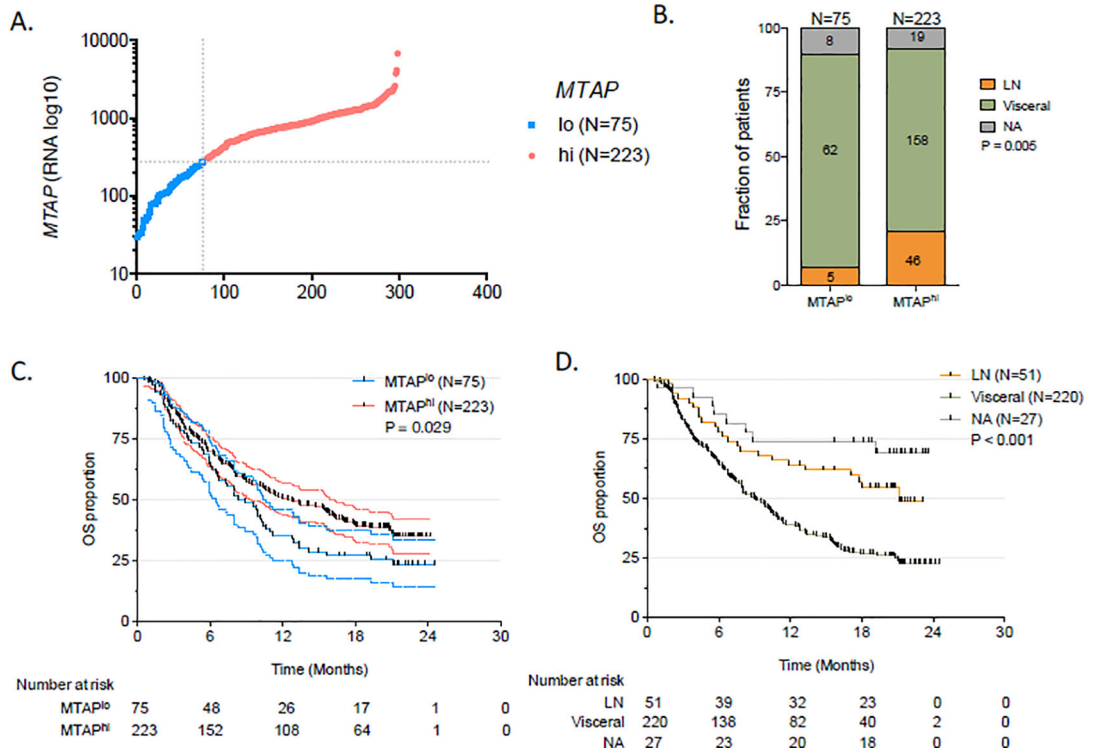
Fig. 2 –.
Correlation between *MTAP* RNA expression, survival, and visceral disease for the validation cohort from the Imvigor210 trial (NCT02951767). (A) *MTAP* mRNA expression status by number of patients plotted on the *x*-axis. (B) Metastatic disease in patients with MTAP^lo, MTAP^hi, and the overall cohort; (C) OS for all patients stratified by *MTAP* expression. (D) OS for all patients stratified by metastatic sites. OS = overall survival; MTAP^lo = MTAP-low; MTAP^hi = MTAP-high; LN = lymph node; NA = not available.