Adverse Histology, Homozygous Loss of CDKN2A/B, and Complex Genomic Alterations in Locally Advanced / Metastatic Renal Mucinous Tubular and Spindle Cell Carcinoma

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Abstract

Mucinous tubular and spindle cell carcinoma (MTSCC) is a rare subtype of renal cell carcinoma with characteristic histologic features and chromosomal alterations. Although typically indolent, a small subset of cases has been reported to exhibit aggressive clinical behavior. We retrospectively identified 33 patients with MTSCC, consisting of 10 cases of locally advanced / metastatic-MTSCC (pT3 or N1 or M1) and 23 kidney confined-MTSCC (pT1/T2) without disease recurrence or progression. Utilizing a single nucleotide polymorphism array and a targeted next-generation sequencing platform, we examined genome-wide molecular alterations in 24 cases, including 11 available samples from 8 patients with locally advanced / metastatic-MTSCC. Ten patients with locally advanced / metastatic-MTSCC were 8 females (80%) and 2 males (20%). At nephrectomy, 7 of these 10 cases (70%) were pT3 or pN1 while the remaining 3 (30%) were pT1/T2. Eight patients (80%) developed metastases and common sites included lymph node (4, 40%), bone (4, 40%), and retroperitoneum (3, 30%). Four patients died of disease (40%) during follow-up. Locally advanced / metastatic-MTSCCs shared typical MTSCC genomic profiles with loss of chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22, while some exhibited additional complex genomic alterations, most frequently a relative gain of 1q (7/8). Homozygous loss of CDKN2A/B was observed in 3 (38%) locally advanced / metastatic-MTSCCs. Tumor necrosis, solid nested/sheet pattern, irregular trabecular/single-file infiltration in a desmoplastic stroma, lymphovascular space invasion, and increased mitotic activity were associated with locally advanced / metastatic-MTSCCs (all p < 0.05). Our findings reveal that MTSCCs with aggressive clinical behavior have progressed through clonal evolution; CDKN2A/B deletion and additional complex genomic abnormalities may contribute to this process. Recognizing the morphologic presentation of high...
grade MTSCC and evaluating adverse histologic features seen in these tumors can help establish a definitive diagnosis and stratify patients for treatment and prognostication.

**Keywords**

mucinous tubular and spindle cell carcinoma; *CDKN2A/B*; clonal evolution; locally advanced; metastatic

**Introduction:**

Mucinous tubular and spindle cell carcinoma (MTSCC) is a rare subtype of renal cell carcinoma with characteristic histological features and indolent clinical behavior. MTSCCs were initially recognized based on their unique morphological features: an admixture of low-grade elongated tubules and bland spindle cells in a mucinous or myxoid stroma. They have also been shown to harbor recurrent chromosomal abnormalities, in particular, loss of chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22, while lacking trisomy of chromosomes 7 and 17. Recent genome-wide studies using single nucleotide polymorphism (SNP) array or next-generation sequencing (NGS) have further confirmed this chromosomal copy number alteration pattern, which serves as a helpful ancillary tool to distinguish MTSCC from papillary renal cell carcinomas (RCC) with overlapping morphologic and immunohistochemical features.

While most studies in the literature suggest MTSCC to be non-aggressive, there have been sporadic reports of MTSCCs demonstrating high-grade features and, occasionally, adverse clinical outcome. Sarcomatoid transformation is the most well-established form of high grade transformation, with the tumor exhibiting a spindle cell component with marked nuclear pleomorphism, increased mitotic activity, frank tumor necrosis, and expansile growth. In addition, some MTSCCs have been reported to present with a high-grade epithelial component. Rare cases of MTSCCs with conventional low-grade morphology have also been reported to eventually develop extra-renal disease. While these reports offer a glimpse of the features that may be associated with aggressive tumor behavior, there have been no studies to date systematically comparing a large series of locally advanced / metastatic-MTSCCs to kidney confined-MTSCCs.

The rapid advances in cancer genomics and the increasing availability of molecular diagnostics testing have generated an unprecedented amount of genetic information linking molecular alterations to prognosis and treatment. These methods also have granted us the ability to screen and classify cases based on their molecular attributes when the morphologic features alone are insufficient. This approach has been particularly effective for RCC with high-grade, non-specific architectural and/or cytologic features, including high-grade MTSCCs, where the characteristic bland tubules and spindle cells may not be apparent.

In this study, we aimed to investigate the histologic and molecular composition of locally advanced / metastatic-MTSCCs by comparing them to kidney confined-MTSCCs without evidence of disease progression to better understand the histologic features and underlying molecular alterations associated with adverse tumor behavior. To our knowledge, the current
study is the largest to date to perform a comprehensive morphologic and molecular analysis of locally advanced / metastatic-MTSCCs.

Materials and Methods:

Case Selection and Histology Review

Our study was approved by the respective Institutional Review Boards from participating institutions. Surgical pathology archives of two institutions were searched for cases of MTSCC diagnosed between the years 2005 and 2018. All archived materials from identified cases were retrieved and centrally re-reviewed by two genitourinary pathologists (C.Y. and Y.C.) to confirm the diagnosis. The possibility of other established histologic subtypes such as papillary RCC, fumarate hydratase (FH)-deficient RCC, succinate dehydrogenase (SDH)-deficient RCC, or MiT family translocation RCC was excluded by ancillary immunohistochemical and/or molecular studies. Three cases of papillary RCC with histologic features mimicking MTSCC and one SDH-deficient RCC were identified and excluded. Two cases initially diagnosed as “unclassified RCC” with diagnostic comments describing focal MTSCC features were included. Twenty-six of the total 33 cases in the cohort represented consecutive cases diagnosed at one institution (Memorial Sloan Kettering Cancer Center), including 7 patients with paired primary and metastatic tumors available for histologic review. Eleven cases with classic morphology, 7 cases with high-grade morphologic features, and 1 case with sarcomatoid transformation were reported in previous studies\textsuperscript{11,16,22}. The 8th edition AJCC TNM system was used for tumor staging. Clinical information for each case was extracted via retrospective chart reviews. Cases with extra-renal involvement (pT3, pT4, N1, or M1) at nephrectomy or during follow-up were considered as locally advanced / metastatic-MTSCC, while cases without any extra-renal involvement at nephrectomy or during follow-up were kidney confined-MTSCC. Detailed histologic characteristics, including the presence or absence of classic MTSCC morphology, infiltrative borders, growth patterns of epithelial component (tubular, tubulopapillary, solid nested/sheet, irregular trabecular/single-file infiltration in desmoplastic stroma), tumor necrosis, vascular invasion (renal vein/renal sinus vein and/or small lymphovascular space invasion), sarcomatoid transformation (defined as an expansile growth of spindle cells with marked nuclear pleomorphism and increased mitotic activity while lacking intermixed epithelial component), WHO/ISUP grade, and mitotic activity were also recorded for each case.

DNA Sample Preparation

Representative areas of the tumors on hematoxylin-and-eosin slides from all cases were selected and macrodissected for molecular analyses. DNA samples were extracted from formalin-fixed paraffin-embedded (FFPE) tissue using QIAamp DNA FFPE tissue kit according to the manufacturer’s standard protocol (Qiagen, Valencia, CA) or using a magnetic bead-based chemagic FFPE DNA Kit (PerkinElmer, Waltham, MA) on a Hamilton chemagic STAR liquid handling system (Hamilton Company, Reno, NV). Concentration and quality of the sample were assessed with Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and gel electrophoresis using reference DNA as a control.
Single Nucleotide Polymorphism (SNP)-array Analysis

DNA samples were analyzed by SNP array using Affymetrix OncoScan CNV Assay (Therm Fisher, Waltham, MA) as previously described\textsuperscript{11,22}. The assay enables the detection of genome-wide copy number alterations such as gain and loss, allele-specific changes including copy neutral loss of heterozygosity (CN-LOH), ploidy, mosaicism, etc. Briefly, 80ng of genomic DNA samples were hybridized to MIP probes followed by gap filling with AT/GC. After removing the unligated probes through exonuclease treatment, the cleavage enzyme was added to linearize the gap-filled circular MIP probes. This was followed by amplification, enrichment, digestion, and hybridization. The hybridized array was washed, stained, and scanned through GENECHIPScanner-7G (Therm Fisher). OncoScan SNP array data were analyzed by the software couple of OncoScan Console ChAS 4.0 (Thermo Fisher Scientific, Waltham, MA) and Nexus Copy Number 10 (BioDiscovery, El Segundo, CA) using Affymetrix TuScan algorithm (Thermo Fisher Scientific, Waltham, MA). All array data were also manually reviewed for subtle alterations not automatically detected. This analysis was performed in a total of 23 cases, including 7 locally advanced / metastatic-MTSCCs and 16 kidney confined-MTSCCs.

Fluorescence in situ Hybridization (FISH) Analysis

FFPE tissue sections of 4 μm thickness with marked tumor areas were used for FISH analysis. Tissue processing, hybridization, post-hybridization washing, and counterstaining were following standard laboratory protocols. To enumerate relevant gene copy levels, FISH probes for 1p36 (labeled in orange) and 1q25 (labeled in green) (from Abbott Molecular, Des Plaines, IL), and the centromere region of chromosome 12 (CEP12, labeled in green, from Abbott Molecular) and MDM2 (12q15, labeled in orange, from Agilent, Santa Clara, CA) were used. Signal analysis was performed in combination with morphology correlation, and at least 100 interphase cells within the marked tumor area were evaluated and imaged using a Zeiss fluorescence microscope coupled with Metasystems ISIS software (Newton, MA).

Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)

MSK-IMPACT, an Illumina-based Hybrid Capture Next Generation Sequencing (NGS) platform, for somatic mutations in up to 468 cancer genes as previously described\textsuperscript{26}, was performed for a total of 7 specimens from 5 patients, including 4 patients with locally advanced / metastatic-MTSCC. The list of genes covered in the platform is provided in Supplementary Table 1. The allele-specific copy number analysis of MSK-IMPACT data was conducted using open-source FACETS (Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing) tool\textsuperscript{27}.

Statistical Analysis

Clinicopathologic parameters were compared using Fisher’s exact and Mann-Whitney U tests. Statistical analysis was conducted using R 3.3.2 (https://www.R-project.org/).
**Results:**

**Clinicopathologic Characteristics**

Among the 33 cases in our patient cohort, 7 cases exhibited extrarenal disease at the time of nephrectomy, including 4 with distant metastasis. Three additional cases although confined to the kidney at resection subsequently developed distant metastases. We grouped these 10 (30%) cases as locally advanced / metastatic-MTSCC and their detailed clinical and pathologic features are summarized in Table 1 and Table 2, respectively. The remaining 23 (70%) kidney-confined MTSCC cases that had no evidence of disease recurrence/progression after nephrectomy were used as the control group (referred to as “indolent MTSCC” hereafter) (Supplementary Table 2 and Supplementary Figure 1).

The comparison of various clinicopathological features between 10 cases of locally advanced / metastatic-MTSCC and 23 cases of indolent MTSCC is summarized in Table 3. Clinically, there was no significant difference in the gender and age distribution of patients between the two groups (p = 0.69 and p = 0.14, respectively). Patients with locally advanced / metastatic-MTSCC showed a 4:1 female to male ratio with a median age of 65.5 years (range 46–71 years). Tumor size for locally advanced / metastatic-MTSCC was larger than indolent MTSCC (p = 0.07), with a median tumor size of 7.8 cm (range 2.7 to 14.0 cm) for locally advanced / metastatic-MTSCC compared to 4.2 cm (range 1.3 to 16.5 cm) for indolent MTSCC. Eight of the 10 (80%) patients in the locally advanced/metastatic group developed metastatic disease, and common sites included lymph node (4, 40%), bone (4, 40%), and retroperitoneum (3, 30%). During a median follow-up period of 26 months (range 1–60 months), 4 patients died of disease (40%), 4 were alive with residual disease (40%), and 2 showed no evidence of residual disease (20%) at last follow-up. In comparison, the median follow-up time was 53 months (range 3–127 months) for patients in the indolent MTSCC group, all had no evidence of disease progression at last follow-up.

Morphologically, in the primary tumors of locally advanced / metastatic-MTSCCs (Table 2 and Figures 1–3), the classic MTSCC-like growth pattern, tightly packed elongated or short tubules merging with a spindle cell element, was identified in all 10 (100%) cases, serving as a clue for considering MTSCC in the differential diagnosis. However, in 8 (80%) cases, instead of bland cytology and low nuclear grade of the classic MTSCC, scattered or more diffusely distributed neoplastic cells in these areas were WHO/ISUP grade 3 (Figures 1B, 2A, and 3A). Infiltrative borders were seen in 7 cases (70%), often as multinodular infiltration into adjacent renal parenchyma (n=4) or as infiltrating tubules/cells extending into perirenal or hilar fat (n=5) (Figure 1). The dominant growth pattern of the epithelial component was tubular (8/10, 80%). Single-file tumor cell infiltration within a desmoplastic stroma was seen in 5 (50%) cases, the solid pattern was seen in 7 (70%), and sarcomatoid transformation was seen in 2 cases (20%). Additionally, necrosis was identified in 7 cases (70%) whereas vascular invasion was present in 4 (40%), including 3 with lymphovascular space invasion and 1 with renal vein invasion. Eight (80%) locally advanced / metastatic-MTSCCs showed high WHO/ISUP grade (grades 3 or 4), and the median mitotic count was 8 per 10 high power fields (HPF) with a range from 1 to 12. Interestingly, 2 (20%) cases
with classic MTSCC morphology, cases #3 and 5, developed metastasis 11 and 17 months after nephrectomy, respectively.

When compared to the histological features of the indolent MTSCC group, necrosis, adverse growth patterns including solid and single-file tumor cells, as well as vascular invasion and increased mitotic counts, showed significant difference (Table 3). Notably, while sarcomatoid transformation is, without doubt, an adverse histologic feature, this feature was only present in 2 cases, precluding the detection of a significant association. Moreover, 7 cases (36.8%) of indolent MTSCC showed infiltrative borders, but mainly as focal, irregular extension into tumor pseudocapsule or adjacent parenchyma, in comparison to the fat or multinodular infiltration in the locally advanced/metastatic group. Among cases in the indolent MTSCC group graded as WHO/ISUP grade 3, the large majority (10 of 13, 77%) exhibited the grade 3 nuclear features only focally.

At the metastatic sites, among the 7 cases examined in this study, 3 tumors exhibited a poorly-differentiated carcinoma or adenocarcinoma morphology (Figures 1G, 2E, and 2G); one was sarcomatoid; 2 cases demonstrated classic MTSCC histologic features but with focal pseudopapillary or papillary features (Figure 3B); one displayed an adenocarcinoma appearance with focal micropapillary features while the primary tumor was consistent with classic MTSCC (Figure 3C-D).

Immunohistochemistry studies performed at the time of diagnosis for the locally advanced/metastatic group showed variable results for commonly used stains: CK7 showed patchy or focal immunoreactivity in 5 of 8 (63%) cases in which the staining was performed and was negative in the other 3 (37%); AMACR was positive in 5 of 6 (83%); CD10 showed focal positivity in 2 of 3 cases; high molecular weight cytokeratin (34\(\beta\)E12), p63, and WT1 each was negative in 2 of 2 cases. All 5 cases with PAX8 staining were positive.

**Cytogenetics and Molecular Characteristics**

Copy number variation analysis was available for 8 patients with locally advanced/metastatic-MTSCC and 16 cases of indolent MTSCC (Figure 4A). Locally advanced/metastatic-MTSCCs shared typical MTSCC genomic profiles, including copy loss or copy neutral loss of heterozygosity (CN-LOH) in chromosomes 1p (8/8, 100%), 4 (6/8, 75%), 6/6q (6/8, 75%), 8 (1/8, 13%), 9 (5/8, 63%), 13 (6/8, 75%), 14 (6/8, 75%), 15 (8/8, 100%), and 22 (7/8, 88%), supporting their classification as MTSCC. Compared to indolent MTSCCs, additional complex genomic alterations were identified, including relative gain of chromosome 1q (n=7; 88% vs. n=0), losses or CN-LOH of chromosomes 3 or 3p (n=6; 75% vs. n=1; 6%), 18 or 18q (n=4; 50% vs. n=1; 6%), 19 (n=3; 38% vs. n=1; 6%), and 21 (n=4; 50% vs. n=3; 19%). In contrast to a lack of gains in the indolent MTSCCs, gains of various chromosomal arms were observed in 8 (80%) samples from 7 (88%) patients with advanced/metastatic MTSCC, most frequently involving 2 (n=4), 7 (n=3), 12 (n=4), and 20 (n=3). Loss of heterozygosity (LOH) was also observed along with some of these additional genomic gains.

In case #3 where we compared the lymph node metastasis with renal primary showing classic morphology by SNP array, the metastasis showed additional losses of chromosomes...
10, 11, and 19, relative gain (CN-LOH) of 1q, as well as complex alterations of 3, 5, 17, and 20, indicative of clonal evolution (Figure 4A and Supplementary Figure 2). Notably, in 3 (38%) cases of the locally advanced / metastatic-MTSCC (cases # 1, 7, and 8), loss of both copies of the CDKN2A/B at 9p21.3 was observed, due to loss of chromosome 9 combined with deletion of 9p21.3 in the other allele or homozygous deletion of 9p21.3 (Figure 4B). Additionally, focal amplification of MET (7q31) and EGFR (7p11.2) were also observed in one case (Case #7). Abnormalities associated with CDKN2A/B, MET, and EGFR were not observed in any of the indolent MTSCCs. Moreover, the metastatic tumor of case #1 showed complex genomic alterations, consistent with the presence of a whole-genome doubling event occurring between initial chromosomal losses and additional copy number changes. This finding was corroborated by FISH results revealing tetraploidy of chromosomes (e.g. Chr 1q and 12) with balanced allele difference in a dominant subpopulation of tumor cells (Figure 4C and Supplementary Figure 3).

No recurrent somatic mutations were seen in the 4 cases of locally advanced / metastatic-MTSCC in which next-generation sequencing was performed. Oncogenic mutations of NF2, KMT2C, EP300, and FBXW7 were detected in one case each. In one case where we compared the two metastases with the renal primary (Case #2), the metastases and primary shared 6 of 8 (75%) somatic mutations, supporting clonal evolution (Supplementary Table 3). Interestingly, as previously reported, one case (case #6) had MLH1 deep deletion and was confirmed to have MLH1 and PMS2 protein loss by immunohistochemistry, which could have therapeutic significance.

Discussion:

We identified 10 cases of locally advanced / metastatic-MTSCC and investigated their morphological, cytogenetics, and molecular features, in comparison to 23 cases of kidney-confined, indolent MTSCC. To our knowledge, this is the first study to systematically study MTSCCs that present with extrarenal disease or show aggressive clinical behavior. We identified adverse histologic features associated with aggressive clinical course and demonstrated molecular evidence to support these tumors as a high grade transformation of MTSCC via clonal evolution, with molecular alterations often associated with aggressive diseases, such as CDKN2A/B deletion and complex copy number alterations.

Previous studies focusing on the prognosis of MTSCCs, mostly case reports, have generated variable results. In 7 cases reported with sarcomatoid transformation, the mean patient age was 71 years (range 64 to 80 years), with female to male ratio of 1:1. On an average of 8 months (range 1 to 19 months) follow-up, 5 (71%) patients developed metastatic disease, while 4 (57%) died of the disease. In 5 cases reported with epithelial high nuclear grade, the mean patient age was 70 years (range 60 to 82 years), with all patients being male. With an average of 18 months (range 4 to 48 months) follow-up, 4 (80%) patients died from metastatic disease. In 3 case series reporting 18 patients with epithelial high nuclear grade, none developed recurrence or metastasis at median 29.2 months follow-up. In addition, advanced disease has even been associated with cases that harbor no adverse histology. However, most case studies with reported adverse outcomes did not have cytogenetics available for confirmation.
of the diagnosis, and even in cases with this data, the chromosomal alterations were not classic. The high prevalence of advanced MTSCCs reported in male patients in the literature is also somewhat controversial considering the female preponderance of classic MTSCC.

Given the morphologic overlap of MTSCC with other subtypes of RCC and limited knowledge regarding MTSCC with aggressive behavior, all cases of locally advanced / metastatic-MTSCC included in this study with available material were examined molecularly to corroborate the pathologic diagnosis. By genome-wide SNP array analysis, they largely demonstrated the frequent chromosomal losses seen in typical MTSCC, supporting their classification as MTSCC. Compared to MTSCC with classic morphology, these tumors often exhibited histologic features commonly associated with aggressive tumor types, including high mitotic rate, tumor necrosis, and lymphovascular invasion, as well as unusual architectural patterns such as single file infiltration and solid growth, all of which were found to be significantly associated with worse outcome in our cohort. Sarcomatoid transformation was found in locally advanced / metastatic-MTSCCs, but only present in 20% of the cases. Tumor infiltrative borders, especially a multinodular infiltration pattern, showed a trend to association with an aggressive clinical course. In comparison, high WHO/ISUP nuclear grade did not always correlate with adverse clinical outcomes, a phenomenon also alluded to in previous studies. Two of our cases and a few prior reports highlighted the existence of a small number of MTSCCs with low nuclear grade but progression to metastatic disease. One strength of our study is that we were able to compare the morphologic features of paired primary and metastatic tumors in a large majority of cases of the locally advanced / metastatic-MTSCC group, demonstrating not only the morphologic spectrum of the primary tumor but also the morphologic evolution at metastatic sites.

Molecularly, while sharing many of the chromosomal losses characteristic of indolent MTSCC, the locally advanced / metastatic-MTSCCs harbored additional genomic alterations not commonly seen in indolent tumors. Most notably, relative gain of chromosome 1q was seen in 7 (7/8, 88%) locally advanced / metastatic-MTSCC cases while not observed in any of the cases in the indolent group. Additional findings that were more commonly seen in locally advanced / metastatic-MTSCCs included loss or CN-LOH of chromosome 3 or 3p (75%), loss of chromosome 18 or 18q (50%), loss of chromosome 19 (38%), loss of chromosome 21 (50%), and chromosomal gains most frequently involving chromosomes 2, 7, 12, and 20. These additional alterations indicate clonal evolution and appear to be associated with more aggressive clinical behavior. Interestingly, while gains of chromosomes 7 and 17q were identified, these were not necessarily associated with papillary architecture. Nonetheless, the complex chromosomal alteration patterns detected in some of these cases highlight the challenge of classifying RCC with high grade features if only using limited cytogenetic or copy number analysis. This could be particularly error-prone for tumors with complex copy number alterations generated by whole-genome doubling, a common phenomenon observed in advanced cancers.

The most common chromosomal alteration detected in locally advanced / metastatic-MTSCC, gain of 1q, has been associated with many cancer types, in particular, Wilms
tumor\textsuperscript{32} and multiple myeloma\textsuperscript{33}. In both scenarios, the presence of 1q gain indicates a worse clinical outcome. Loss of chromosome 1p has also frequently been associated with worse prognosis in various non-kidney tumor types\textsuperscript{34,35}. Multiple tumor suppressor genes are located on chromosome 1p\textsuperscript{35,36}, while tumor oncogenes are located on chromosome 1q\textsuperscript{37,38}, thus an unbalanced loss of 1p (or relative gain of 1q) could theoretically result in tumor progression.

We also demonstrated homozygous loss of $CDKN2A/B$ in 3 (38\%) of our locally advanced / metastatic-MTSCC cases, which was not observed in any of our indolent MTSCC cases. While representing a novel finding for MTSCC, molecular alterations of $CDKN2A/B$ leading to a loss of function have been well documented in many tumor types and are a negative prognostic factor, including in common histologic subtypes of RCC\textsuperscript{39,40}. In the current study, the cases with homozygous loss of $CDKN2A/B$ also exhibited adverse morphologic features, including significantly increased mitotic index (8–12/10 HPF) and necrosis. Recently, the frequent co-deletion of the neighboring $MTAP$ gene has been suggested to provide a vulnerability for targeting tumors with homozygous loss of $CDKN2A/B$, implying the potential of using this molecular feature to stratify patients for therapeutic approaches\textsuperscript{41,42}.

In two cases of locally advanced / metastatic-MTSCC, we had sufficient material for molecular studies in both the primary and metastasis. In one case, three separate tumor samples (taken from the kidney, vena cava, and retroperitoneum) analyzed by NGS showed similar chromosomal copy number alterations and shared a large majority of detected somatic mutations, indicating a common origin and clonal evolution. The other patient presented with a large but localized classic MTSCC and subsequently developed metastatic disease. By SNP array analysis, while the losses of chromosomes 1, 4, 6, 9, 13, 14, 15, 18, 21, and 22 seen in the primary tumor were mostly preserved in the lymph node metastasis, multiple additional chromosomal losses and gains were found. Interestingly, these additional changes were also associated with a morphologic shift from classic MTSCC morphology to a non-specific adenocarcinoma appearance.

For four cases of locally advanced / metastatic-MTSCCs sequenced by our targeted sequencing panel, we did not identify recurrent somatic mutations. Mutations of $NF2$, $KMT2C$, $EP300$, and $FBXW7$ were detected in one case each. Biallelic alterations and dysregulation of the Hippo signaling pathway, including mutations of $NF2$ gene, have been identified with MTSCCs\textsuperscript{12}. While our study suggests a lack of common mutations underlying the aggressive behaviors of these MTSCC cases, the presence of mutations in tumor suppressor genes including $KMT2C$, $EP300$, and $FBXW7$ suggests aberrant chromatin modification and protein degradation pathways might be involved in the process. Independent validation in larger cohorts using whole-exome studies would be ideal, yet very challenging from a practical perspective given the rarity of these cases.

Supported by the molecular characterization, we were able to better delineate the morphologic spectrum of MTSCC with aggressive behavior. It is important to recognize that locally advanced / metastatic-MTSCC often lose characteristic morphologic features – admixed low-grade tubules and bland spindle cells – and show morphologic and
immunophenotypic overlap with high grade papillary RCC, collecting duct carcinoma, FH-deficient RCC, SDH-deficient RCC, and other rare histologic subtypes including unclassified RCC, especially in cases that are mucin poor\textsuperscript{43,44}. An important clue for including MTSCC in the differential diagnosis is to recognize the focally preserved MTSCC or MTSCC-like architectural pattern despite the frequently high nuclear grade. To accomplish this, sufficient sampling in nephrectomy specimens is critical. Similar to our previous study of classic MTSCC\textsuperscript{11}, there were no distinct, well-formed type 1 papillary RCC-like areas identified in these high grade MTSCC cases. A definitive distinction from FH- or SDH-deficient RCC requires ancillary studies, which were undertaken in our cohort to exclude these possibilities. While the histologic features seen in some of these cases would fulfill the morphologic diagnostic criteria of collecting duct carcinoma, the molecular evidence provided here and in previous studies support their derivation from classic MTSCC.

For metastatic tumors that were sampled in small biopsies, it is extremely challenging to assign the MTSCC diagnosis solely based on morphologic features. Tumors often present with a non-specific, poorly-differentiated adenocarcinoma morphology, or exhibit a pseudopapillary or papillary architecture. Accurate diagnosis would be important in this scenario but may not be feasible by morphology alone. Genome-wide copy number assessment, ideally utilizing SNP array to identify copy neutral LOH, will be a useful tool for diagnosis based on our analysis. Recent reports of novel ancillary markers, such as VSTM2A, would also be interesting to test in this setting\textsuperscript{45}. On the other hand, routine immunohistochemistry markers like CK7 and AMACR are of limited utility, as similar labeling may be seen in differential diagnostic considerations, including papillary RCC and collecting duct carcinoma.

In summary, we systematically studied a cohort of locally advanced / metastatic-MTSCCs and described their clinicopathologic and molecular features. Adverse histologic features, including necrosis, solid growth, single file infiltration, sarcomatoid transformation, lymphovascular invasion, and increased mitoses are associated with aggressive disease behavior. Complex genomic alterations, such as relative gain of chromosome 1q and homozygous deletion for CDKN2A/B are also enriched in clinically aggressive MTSCCs. MTSCC with high-grade features and aggressive clinical behavior likely have progressed through clonal evolution. Although MTSCCs are, in general, considered to be indolent, we demonstrated that cases of MTSCC with malignant clinical behavior exist, and their diagnosis can be confirmed with cytogenetics and/or molecular studies.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Locally advanced / metastatic mucinous tubular and spindle cell carcinoma-Case #6: A) multinodular infiltrative border at the periphery; B) classic MTSCC-like area, but with scattered WHO/ISUP grade 3 nucleoli and increased mitoses (inset); C) tubular and solid growth with high WHO/ISUP grade 3 cells; D) single-file and irregular trabecular/tubular growth; E) rhabdoid cells; F) fat invasion; and G) metastasis to bone.
Figure 2.
Locally advanced / metastatic mucinous tubular and spindle cell carcinoma-Case #1 (A-E) and Case #7 (F-G). A) classic MTSCC-like area transitioning into WHO/ISUP grade 3 area; B) tubular and tubulopapillary area with focal psammomatous calcification; C) necrosis and solid area; D) sarcomatoid transformation; E) metastasis to epidura, showing features of a poorly differentiated carcinoma; F) representative image of the primary tumor in Case #7, showing admixed WHO/ISUP grade 2 and grade 3 cells (inset); G) metastasis to retroperitoneum, showing features of a poorly differentiated carcinoma.
Figure 3.
Locally advanced / metastatic mucinous tubular and spindle cell carcinoma-Case #2 (A-B) and Case #3 (C-D). A) primary tumor with classic MTSCC-like architecture and WHO/ISUP grade 3 nucleoli; B) metastasis to retroperitoneum with similar morphology but shows pseudopapillary arrangement (black arrow); C) primary tumor without adverse histologic features; however, developed D) metastasis to retroperitoneum one year later that shows tubular and tubulopapillary features.
Figure 4.
A) Copy number alterations detected in each case of mucinous tubular and spindle cell carcinoma (MTSCC). Locally advanced / metastatic-MTSCC cases are shown on top and indolent MTSCC cases are shown below. B) Representative result of a case (#8) showing CDKN2A/B gene homozygous loss. Single nucleotide polymorphism (SNP) array shows homozygous deletion of CDKN2A/B gene locus (arrow) and neighboring areas with heterozygous loss. C) FISH analysis of Case #1 showing two copies of 1p36 (orange) and four copies of 1q25 (green) in 53% of cells (top), three to four copies of chromosome 12.
with three to four copies each for CEP12 (green) and MDM2 (orange) in 60% of cells (bottom), consistent with doubling genomic profilings revealed by SNP array.
Table 1.
Clinical characteristics of locally advanced/metastatic mucinous tubular and spindle cell carcinoma.

| Case | Gender | Age | Laterality | Size (cm) | Stage | Metastasis | Time to Metastasis (months)# | Follow-up (months) | Outcome |
|------|--------|-----|------------|-----------|-------|------------|-------------------------------|-------------------|---------|
| 1    | Male   | 58  | Right      | 2.7       | pT3aNx| Brain; Spinal Cord; Lung; Liver; Soft Tissue (Pelvis) | 12               | 14                 | DOD     |
| 2    | Male   | 59  | Left       | 12.0      | pT3aNXM1| Retroperitoneum; Pleura; Liver; Lymph node | -                | 51                 | DOD     |
| 3    | Female | 67  | Right      | 14.0      | pT2bNx| Retroperitoneum; Lymph node | 11               | 19                 | AWD     |
| 4    | Female | 71  | Left       | 9.5       | pT3aN1M1| Bone; Lung; Lymph node | -                | 4                  | DOD     |
| 5    | Female | 56  | Left       | 11.5      | pT2bNx| Iliac; Vertebral | 17               | 29                 | DOD     |
| 6    | Female | 68  | Right      | 5.1       | pT3aNXM1| Bone; Skin; Retroperitoneum | -                | 23                 | AWD     |
| 7    | Female | 66  | Right      | 14.0      | pT2bNx| Peritoneum | 46               | 49                 | AWD     |
| 8    | Female | 65  | Right      | 6.0       | pT3aN1M1| Vertebral; Lymph node | -                | 1                  | AWD     |
| 9    | Female | 66  | Right      | 3.0       | pT3aNx| -          | -                | 60                 | NED **  |
| 10   | Female | 46  | Left       | 6.0       | pT3aNx| -          | -                | 60                 | NED **  |

DOD - died of disease; AWD - alive with disease; NED - no evidence of disease

* At the time of surgery
# Time between nephrectomy and the first diagnosis of distant metastasis
** Clinical information extracted from previous publication17
Table 2.
Histologic characteristics of locally advanced/metastatic mucinous tubular and spindle cell carcinoma.

| Case | Classic MTSCC-like Area* | Dominant Epithelial Architectural Pattern | Infiltrative Border | Single File | Solid | sarcomatoid transformation | WHO/ISUP Grade | Necrosis | Mitosis (10HPF) | Vascular invasion |
|------|---------------------------|------------------------------------------|---------------------|------------|-------|----------------------------|----------------|----------|-----------------|------------------|
| 1    | Yes                       | Tubular papillary                         | Yes                 | Yes        | Yes   | Yes                        | 4              | Yes      | 8               | No               |
| 2    | Yes                       | Tubular                                  | Yes                 | Yes        | Yes   | No                         | 3              | Yes      | 5               | Yes (renal vein) |
| 3    | Yes                       | Tubular                                  | No                  | No         | No    | No                         | 2              | No       | 1               | No               |
| 4    | Yes                       | Solid                                    | Yes                 | No         | Yes   | Yes                        | 4              | Yes      | 5               | Yes (LVI)        |
| 5    | Yes                       | Tubular                                  | No                  | No         | No    | No                         | 2              | No       | ND              | No               |
| 6    | Yes                       | Tubular                                  | Yes                 | Yes        | Yes   | No                         | 4 (rhabdoid)   | Yes      | 10              | No               |
| 7    | Yes                       | Tubular                                  | No                  | Yes        | Yes   | No                         | 3              | Yes      | 8               | No               |
| 8    | Yes                       | Tubular                                  | Yes                 | No         | Yes   | No                         | 3 (focal)      | Yes      | 12              | Yes (LVI)        |
| 9    | Yes                       | Tubular                                  | Yes                 | No         | No    | No                         | 3              | No       | 1               | No               |
| 10   | Yes                       | Tubular                                  | Yes                 | Yes        | Yes   | No                         | 3              | Yes      | 8               | No               |

* Tumor displays tightly packed elongated or short tubules merging with a spindle cell element; high-grade nuclei are allowed.

LVI: Lymphovascular invasion; HPF: High-power fields; ND: Not determined.
Table 3.
Comparison of clinicopathologic features in the locally advanced/metastatic and indolent Mucinous and Tubular Spindle Cell Carcinoma (MTSCC) groups.

|                      | Locally advanced/metastatic-MTSCC (n = 10) | Indolent MTSCC (n = 23) | p value |
|----------------------|------------------------------------------|-------------------------|---------|
| **Gender**           |                                          |                         | 0.69    |
| Male                 | 2 (20%)                                  | 7 (30%)                 |         |
| Female               | 8 (80%)                                  | 16 (70%)                |         |
| **Age, years**       |                                          |                         | 0.14    |
| Mean                 | 63.8                                     | 58.0                    |         |
| Median               | 65.5                                     | 57.0                    |         |
| Range                | 55–73                                    | 21–82                   |         |
| **Size**             |                                          |                         | 0.07    |
| Mean                 | 8.4                                      | 5.4                     |         |
| Median               | 7.8                                      | 4.2                     |         |
| Range                | 2.7–14.0                                 | 1.3–16.5                |         |
| **WHO/ISUP grade**   |                                          |                         | 0.07    |
| Low (grades 1–2)     | 2                                        | 10                      |         |
| High (grades 3–4)    | 8                                        | 13                      |         |
| **Mitosis (10HPF)**  |                                          |                         | <0.001  |
| Mean                 | 6.4                                      | 0.13                    |         |
| Median               | 8                                        | 0                       |         |
| Range                | 1–12                                     | 0–1                     |         |
| **Necrosis**         | 7/10 (70%)                               | 1/23 (4%)               | <0.001  |
| **Adverse growth patterns** |                                  |                         |         |
| Solid growth         | 7/10 (70%)                               | 3/23 (13%)              | 0.002   |
| Single file infiltration | 5/10 (50%)                             | 1/23 (4%)               | 0.005   |
| Sarcomatoid          | 2/10 (20%)                               | 0                       | 0.09    |
| Infiltrative border  | 7/10 (70%)                               | 7/23 (30%)              | 0.06    |
| Vascular invasion    | 4/10 (40%)                               | 0                       | 0.005   |
| **Stage**            |                                          |                         | <0.001  |
| pT1–2                | 3 (30%)                                  | 23 (100%)               |         |
| pT3–4                | 7 (70%)                                  | 0                       |         |
| **Recurrence/Metastases** |                                  |                         | <0.001  |
| Yes                  | 8 (80%)                                  | 0                       |         |
| No                   | 2 (20%)                                  | 23 (100%)               |         |
| **Survival**         |                                          |                         | 0.005   |
| Alive                | 6 (60%)                                  | 23 (100%)               |         |
| Dead                 | 4 (40%)                                  | 0                       |         |