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Original contribution

High-stage urachal adenocarcinoma can be associated with microsatellite instability and *KRAS* mutations☆

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Summary Urachal adenocarcinoma (UAC) is a rare tumor of the urinary bladder, which can show intestinal, mucinous, and signet ring cell histology. The morphology is similar to that of colorectal adenocarcinoma (CAC). Microsatellite instability (MSI), *KRAS*, and *BRAF* have been more extensively studied in CAC. What is not known is whether UAC in its morphologic similarity to CAC could show immunohistochemical features of MSI along with *KRAS*- and *BRAF*-activating mutations. A retrospective review of institutional archives for UAC cases found 7 cases, all of which were high stage. Most (6/7) of our UAC cases showed evidence of MSI or mutations of *KRAS*. No cases showed a *BRAF* mutation at codon 600. Of the cases that demonstrated MSI, 1 showed mutS homolog 2 and mutS homolog 6 loss, and 2 showed PMS2 (postmeiotic segregation increased 2) loss. Of the remaining 4 cases, 3 showed *KRAS* mutations at codon 12. Our UAC series showed mutual exclusivity of MSI and *KRAS* mutations. Furthermore, our UAC cases with *KRAS* mutations showed markedly better overall survival (mean, 101.7 versus 6.5 months; $P = .035$). Thus, our study justifies ancillary testing for MSI and *KRAS* in UAC, particularly when there is high-stage and mucinous histology, but a larger multi-institutional accruement of UAC cases is necessary to further validate our novel findings.

1. Introduction

Urachal adenocarcinoma is a rare tumor arising from urachal remnants and comprising 0.7% of all urinary bladder cancers. Mainstay of treatment is partial or radical cystectomy with en bloc resection of the medial umbilical ligament up to the umbilicus. Protocols for chemotherapy are non-standardized and institution dependent but frequently involve a 5-fluorouracil and/or platinum-based regimen. Several histologic variants of urachal adenocarcinoma are described and include mucinous, enteric not otherwise specified (NOS), and signet ring cell (SRC) subtypes. The morphology is similar to that of colorectal adenocarcinoma.

KRAS-activating mutations are frequently seen in sporadic colorectal adenocarcinoma. Microsatellite instability (MSI), the molecular fingerprint of the deficient mismatch repair system, is associated more with mucinous and SRC subtypes of colorectal adenocarcinoma. With colorectal adenocarcinoma, MSI can develop as a result of germ line mutations in mismatch repair genes but more commonly from epigenetic silencing of mutL homolog 1 (*MLH1*) in sporadic tumors that frequently show hotspot mutations in the *BRAF* oncogene [1]. What is not known is whether urachal adenocarcinoma in its morphologic similarity to colorectal adenocarcinoma can show immunohistochemical features of MSI along with *KRAS*- and *BRAF*-activating mutations.

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2. Materials and methods

Our study included 7 urachal adenocarcinomas obtained from the Department of Pathology archives at Wake Forest University Baptist Medical Center from 1997 to 2012. The study was approved by the Institutional Review Board at Wake Forest University. These urinary bladder tumors were diagnosed as urachal adenocarcinoma and staged based on published criteria [2]. All specimens were larger resections (partial or radical cystectomy) apart from 1 case, which was obtained from a transurethral resection. In that case, the diagnosis of urachal adenocarcinoma was solidified in tandem with extensive clinical and radiologic workup.

MSI was evaluated by immunohistochemical markers: MLH1, mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and postmeiotic segregation increased 2 (PMS2). Immunohistochemistry was performed on the Leica Microsystems Bond III automated immunostainer (Buffalo Grove, IL). The MLH1 and MSH6 antibodies from Cell Marque Corporation were prediluted antibodies and placed in the Bond Epitope Retrieval solution 2 for 20 minutes. The MSH2 and PMS2 antibodies were from Leica Microsystems, with dilutions of 1:40 and 1:100, respectively, placed in the Bond Epitope Retrieval solution 2 for 20 minutes. Antibody detection was performed using the Bond Polymer Refine detection kit, with DAB (3,3′-diaminobenzidine) as the chromogen and hematoxylin as the counterstain.

KRAS mutation testing was performed by real-time polymerase chain reaction (PCR), using the Qiagen *KRAS* PCR kit on the Applied Biosystems 7500 real-time PCR instrument. This assay was used for the detection of 7 mutations in codons 12 and 13 within a background of normal (wild-type) DNA. The reagents allow for the allele-specific amplification to detect wild-type *KRAS* as well as detect and discriminate between the 7 defined clinically relevant mutations.

BRAF mutation testing at codon 600 was performed by real-time PCR, using a laboratory-developed assay on the Applied Biosystems 7500 real-time PCR instrument. This test is an allele-specific real-time PCR assay that also coamplifies an internal control to identify false negatives.

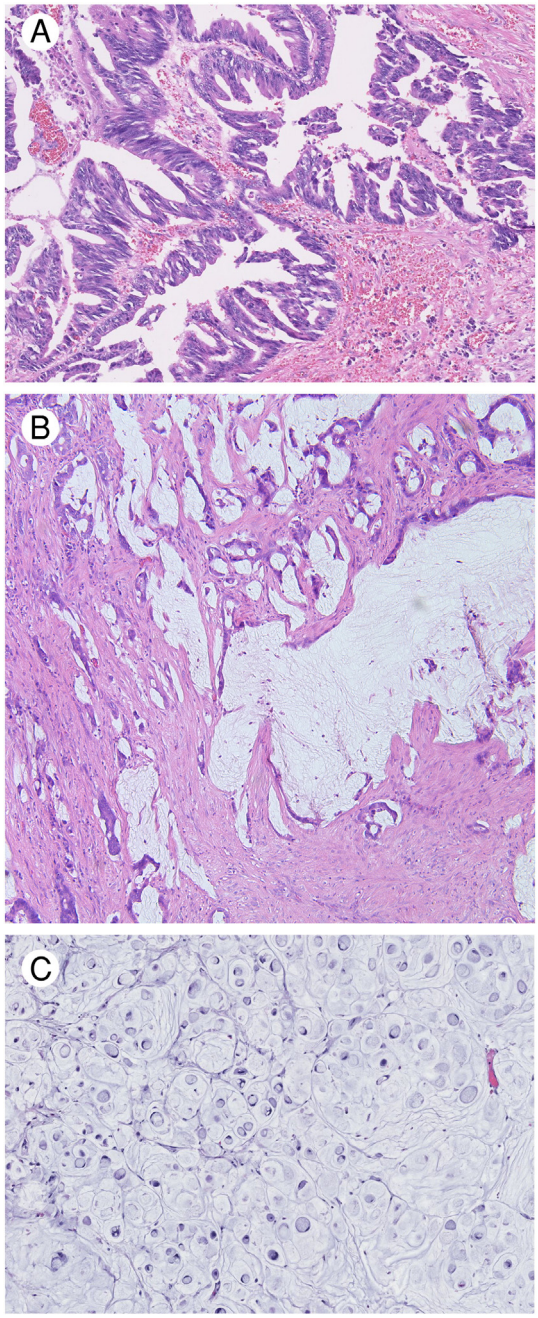

Fig. 1 A, Case 1 is an enteric NOS histologic subtype (hematoxylin and eosin, objective ×20). B, Case 5 is an example of a mucinous histologic subtype (hematoxylin and eosin, objective ×10). C, Case 4 is an example of a mucinous with SRC histologic subtype (hematoxylin and eosin, objective ×40).

3. Results

All urachal tumor cases were adenocarcinomas. One was of enteric NOS histology (Fig. 1A). Six showed mucinous histology, with 2 showing pure mucinous histology (Fig. 1B) and 4 showing additional SRC histology (Fig. 1C). Of those 4 cases, 2 had only focal SRC presence. Patient ages ranged from 18 to 71 years with a median of 54 years. Of the 7 patients, 5 were male (71%). All 7 patients were high stage with 4 patients with pT4 tumors at presentation. The most common metastatic site was the liver (n = 3), followed by brain and bone metastasis. A thorough review of the clinical history showed that 3 patients received chemotherapy containing 5-fluorouracil. One patient received additional platinum-based chemotherapy. None of our patients underwent tyrosine kinase inhibitor anti-EGFR (epidermal growth factor receptor) therapy.

Three cases showed MSI, 1 with MSH2 and MSH6 loss (Fig. 2) and 2 with PMS2 loss. Three cases showed a *KRAS* mutation on codon 12. No case showed a *BRAF* mutation on codon 600. Whether a case showed MSI or a *KRAS* mutation