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RESEARCH ARTICLE

Analysis of Intestinal Metaplasia Without Dysplasia in the Urinary Bladder Reveal Only Rare Mutations Associated With Colorectal Adenocarcinoma

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Abstract: Intestinal metaplasia (IM) is a rare finding in urinary bladder specimens. It is unclear whether IM without dysplasia is a precursor of malignancy in the urinary system. We retrospectively selected 9 cases of IM of bladder (1 case harboring high-grade dysplasia), and performed mutation analysis for genes frequently mutated in colon cancer including *BRAF*, *APC*, *KRAS*, *MET*, *NRAS*, *PIK3CA*, *CTNNB1*, *FBXW7*, and *TP53* using validated clinical tests. Control groups included 7 colonic tubular adenomas, 10 high-grade papillary urothelial carcinomas. One IM case revealed an *APC* mutation and another showed an *NRAS* mutation. Among the tubular adenomas cases, 6 of 7 (85.7%) harbored *KRAS* mutations and 3 of 7 (42%) *APC* mutations. Among urothelial carcinomas cases, 1 revealed had *KRAS* mutation, 2 had *PIK3CA* mutations, and all cases were negative for *APC* mutations. Clinical follow-up for the IM patients was available with a median follow-up of 70 months. One patient—without any mutation in the genes investigated—developed invasive bladder adenocarcinoma with intestinal differentiation with metastasis to the liver and lung. Neither of the 2 patients harboring mutations developed any malignancy. In conclusion, a minority of cases with IM without dysplasia bear mutations in the genes commonly associated with colonic adenocarcinoma, suggesting a premalignant potential for such lesions possibly following the classic multistep chromosomal instability pathway of carcinogenesis. A larger cohort of patients with longer follow-up is needed to better establish whether close follow-up is warranted for mutation-harboring IM of the bladder.

Key Words: intestinal metaplasia, bladder cancer, adenocarcinoma

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Intestinal (mucinous) metaplasia (IM), or metaplastic replacement of urothelium by intestinal type epithelium, is an uncommon finding in the urinary bladder. It is far less common than cystitis cystica et glandularis, which is a benign condition usually related to inflammation or infection. Occasionally, both cystitis cystica et glandularis and IM coexist in the same patient/specimen. When the epithelium manifests colonic phenotype with goblet cell metaplasia, it also acquires an intestinal immunoprofile and may show dysplasia or adenocarcinoma.[1] It is controversial if IM acts as a precursor of malignancy in the urinary system. In this study we investigate the presence of mutations that are commonly identified in colorectal adenocarcinoma in a series of IM of the bladder. For comparison, we also performed the same mutational analysis in adenomas of the colon and high-grade papillary urothelial carcinomas (UC).

MATERIAL AND METHODS

After obtaining pertinent institutional review board approval, a search for IM of the bladder was performed. From 2005 to 2014, 15 cases were retrieved. For control groups, 10 random cases of high-grade UC of bladder, 10 adenomatous lesions of the colon [tubular adenomas (TA)]. The diagnoses were confirmed by 2 GU pathologists (A.A. and A.M.) and the best slide/block was selected for the study. Areas of interest were marked on H&E slides and DNA was isolated from paraffin embedded tissue sections using laser capture microdissection method as described previously or macrodissection from sequentially sectioned unstained slides.[2]

Microdissected sections were incubated in proteinase-K solution overnight at 70°C and then extracted using the Maxwell LEV automated extractor (Promega, Fitchburg, WI). The DNA concentration was determined using the Nanodrop and a quality control assay was performed on each specimen by amplifying different size amplicons from 100 to 600 bp in length (Invivoscribe, San Diego, CA). Mutation analysis for genes commonly involved in colonic adenocarcinoma (*BRAF*, *APC*, *KRAS*, *MET*, *NRAS*, *PIK3CA*, *CTNNB1*, *FBXW7*, and *TP53*) was performed using MassARRAY System (Sequenom Laboratories, San Diego, CA). DNA from tissue samples

from non-neoplastic colonic mucosa previously known to be negative for the mutations tested was used in each run to control for cross contamination. The test was performed in a CLIA certified clinical laboratory of Lifespan Academic Medical Center.

Macrodissected samples were processed in the CLIA certified Molecular Diagnostics Laboratory at the Johns Hopkins Hospital. DNA extraction and purification of macrodissected samples were performed with the automated Siemens Tissue Preparation System (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) and quantified using the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA). The samples were then sequenced on the Ion S5 XL Semiconductor Sequencer using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific Inc., Waltham, MA) for targeted multigene amplification. Sequencing data were analyzed using Torrent Suite and mutations were identified and annotated through the Torrent Variant Caller (Thermo Fisher Scientific Inc.) and by direct visual inspection of the binary sequence alignment/map file using the Broad Institute's Integrative Genomics Viewer (www.broadinstitute.org/igv/).

Clinical follow-up was obtained through retrospective review of the medical records.

RESULTS

Intestinal Metaplasia of the Bladder

The IM group included 15 cases, 11 males and 4 females, with mean age of 50 years (range, 18 to 87 y) (Table 1). Clinical presentations included hematuria (n = 7), outlet obstruction (n = 2), and surveillance or resection for UC (n = 6). The relative quantity of the tissue with IM varied between 1% and 20% of the total specimen. DNA content was considered inadequate for assessment in 6 cases, therefore, only 9 of the 15 cases were included in the final mutational analysis. Among the patients undergoing surveillance for clinical history of UC, 2 cases showed mutations in either *APC* (p.R232 in 10.5% of the lesional cells) or *NRAS* (p.G13D in 13% of the lesional cells). The remaining 7 (78%) cases had no detectable hotspot mutations in any of the genes assessed (Table 1).

Clinical follow-up for the IM patients was available with a median follow-up of 70 months. One patient—without any mutation identified—developed invasive bladder adenocarcinoma with intestinal differentiation. This patient developed metastasis to liver and lung. Neither of the 2 patients harboring mutations developed any malignancy during follow-up.

There was 1 case included in the study that revealed IM with high-grade dysplasia in a tubulovillous adenoma of the bladder, with a focus suspicious, but not diagnostic, of lamina propria invasion (case#1). The case did not reveal any mutation in the panel assessed. The morphologic spectrum of lesions with IM is presented in Figure 1.

Urothelial Carcinoma Group

The UC group included 10 cases, 9 males and 1 female, with mean age of 69.3 years (range, 50 to 83 y) (Table 2). Clinical presentations included hematuria in 5 cases, and surveillance for high-grade UC in 5 cases. All patients underwent transurethral resection of bladder tumor (TURBT), and the specimens revealed high grade UC (no variant morphology was identified). Five cases revealed superficial invasion into lamina propria, 2 showed foci suspicious (but not definite) for invasion, 2 were noninvasive carcinomas, and 1 showed invasion into muscularis propria.

The mutation analysis was successful in all cases, 8/10 (80%) cases showed no mutation in the genes assessed. Two cases revealed mutation in *PIK3CA*, one with a concomitant mutation in *TP53* and the other with concomitant mutation in *KRAS* (Table 2). All cases were negative for *APC* mutation.

Colonic Adenoma Group

The TA group included 10 specimens, 6 males and 4 females, with mean age of 63.4 years (range, 50 to 83 y) (Table 3). All cases were resected for colonic polyp (size range

TABLE 1. Demographics and Mutational Analysis of Intestinal Metaplasia Cases

Case #	Sex	Age (y)	Location	Diagnosis	Mutation (MAF)
1	M	48	Bladder neck	IM, bladder TVA with high grade dysplasia	—
2	M	73	Bladder, lateral wall	IM, Urothelial CIS	—
3	M	30	Bladder trigone	IM, polypoid cystitis	—
4	M	49	Bladder NOS	IM	*NRAS* G13D (13%)
5	F	33	Bladder neck	IM, endometriosis	—
6	F	59	Bladder, posterior wall	IM, polypoid cystitis	—
7	F	70	Bladder trigone	IM, polypoid cystitis	—
8	M	31	Bladder neck	IM, NA	*APC* R232 (10.2%)
9	F	18	Bladder, posterior wall	IM	N/A
10	M	87	Bladder, posterior wall	IM	N/A
11	M	27	Bladder NOS	IM, NA	N/A
12	M	62	Bladder trigone	IM	N/A
13	M	39	Bladder trigone	IM with extravasated mucin	—
14	M	53	Bladder trigone	IM, polypoid cystitis	—
15	M	71	Bladder, diverticulum	IM	N/A

CIS indicates urothelial carcinoma in situ; F, female; IM, intestinal metaplasia; M, male; MAF, mutant allele frequency; N/A, not available; NA, nephrogenic adenoma; NOS, not otherwise specified; TVA, tubulovillous adenoma.

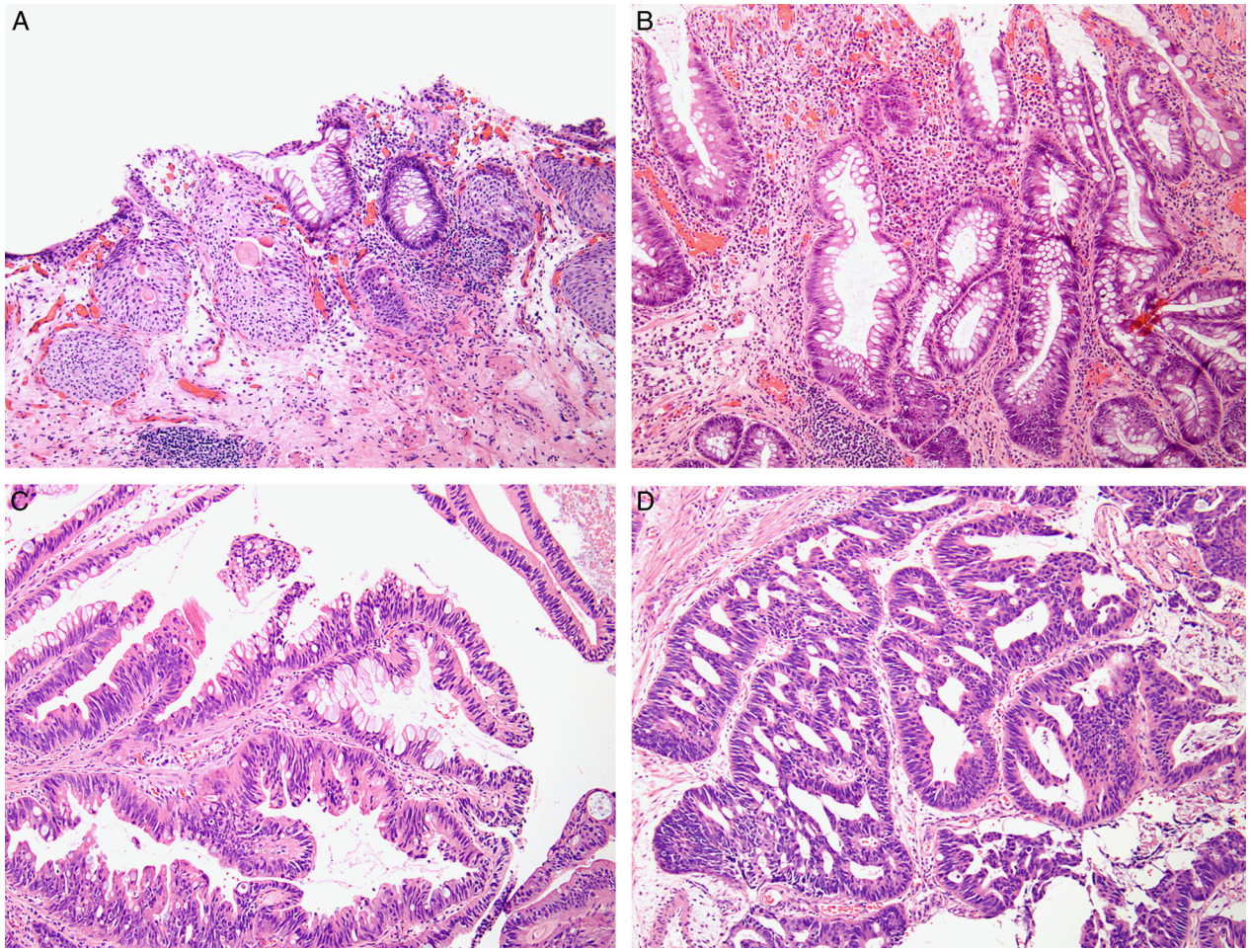

FIGURE 1. Spectrum of findings in patients with intestinal metaplasia of the bladder. A, Benign urothelium with cystitis cystica et glandularis and a focus of intestinal metaplasia without dysplasia. B, Focus of intestinal metaplasia without dysplasia and without association with cystitis cystica glandularis. C, Intestinal metaplasia with low-grade dysplasia. D, A case of adenocarcinoma with intestinal differentiation arising in association with intestinal metaplasia.

between 1.8 and 6.2 cm). Two cases showed high-grade dysplasia, without any evidence of invasive carcinoma in any case.

The mutation analysis was successful in 7 cases, and mutations were identified in 6 cases (85.7%). All cases with mutation revealed various mutations including *KRAS*. Most common *KRAS* mutations were at p.G12D (3 cases, 50%), with the rest of the cases showing mutation in p. G12S, p.G13D, and p.A146T (1 case each). In addition, 3 cases revealed mutations in *APC* (42.8%), located in p. E1309 (in 2 cases) and p.R1114 (1 case). Two cases revealed mutations in *FBXW7* (28.5%) and 1 case had mutation in *CTNNB1*. One case with TA of ileocecal valve did not harbor any mutation in the genes evaluated.

DISCUSSION

IM in bladder is more frequent around the trigone, and the patients usually complain from hematuria, or less common urinary symptoms like mucosuria.[3] There is controversy in the prognosis and outcome of IM. Some studies suggest that IM is a premalignant lesion because it is usually identified adjacent to concurrent adenocarcinoma of bladder; however, many IM cases do not develop into adenocarcinoma.[3]

A study by Kao and Epstein[4] that included a short series of TA of the bladder showed that they have the same immunophenotype as the adenomas of the gastrointestinal tract, they are positive for CK20 and CDX2, whereas negative for GATA3 and CK7. One case showed positive nuclear beta-catenin, similar to that seen in colonic adenocarcinoma.[5] This study suggested that some of the lesions follow a molecular pathway of carcinogenesis seen in colon.

A more recent study by Gordetsky and Epstein included 19 patients with IM with dysplasia, 8 patients developed concurrent adenocarcinoma of bladder. Of the 11 patients who did not develop concurrent adenocarcinoma, none revealed persistent/recurrent dysplasia. Only 1 patient developed high-grade UC in the follow-up. The authors concluded that a significant subset of patients with IM with dysplasia will develop adenocarcinoma and thus recommend clinical follow-up of such lesions.[6]

TABLE 2. Demographic Findings of High-grade Urothelial Carcinoma Cases and Their Mutations

Case #	Sex	Age (y)	Location	Diagnosis	Mutation (MAF)
1	M	62	Bladder NOS	HGSUSP	—
2	M	68	Prostatic urethra	HGTCC	—
3	M	69	Bladder, left wall	INVPAP	—
4	F	81	Bladder NOS	INVPAP	—
5	M	79	Bladder, dome	INVPAP	*PIK3CA* E545K (34.3%) *TP53* (32.8%)
6	M	87	Bladder NOS	INVPAP	*KRAS* G13D (46.1%) *PIK3CA* E545K (49.5%)
7	M	46	Bladder NOS	INVPAP	—
8	M	57	Bladder NOS	HGSUSP	—
9	M	65	Bladder, left wall	INVPAP	—
10	M	79	Bladder NOS	HGTCC	—

F indicates female; HGSUSP, high-grade papillary urothelial carcinoma, suspicious for early lamina propria invasion; HGTCC, noninvasive high-grade papillary urothelial carcinoma; INVPAP, invasive high-grade papillary urothelial carcinoma; M, male; MAF, mutant allele frequency; NOS, not otherwise specified.

Morton and colleagues, evaluated 34 patients with IM and noted reduced average telomerase signal intensity, suggestive of shortening of telomerase length. When cystitis cystica glandularis was present for comparison, there was significantly lower signal intensity in IM compared with cystitis cystica. The authors concluded that IM is a precursor lesion and can give rise to bladder adenocarcinoma and that the telomerase length in cystitis cystica glandularis was intermediate between normal and IM.[7] This finding was interpreted as a potential for cystitis cystica to develop into IM and eventually adenocarcinoma.

Bryan and colleagues studied beta-catenin and TNF-alpha expression in IM and cystitis cystica glandularis using immunohistochemistry and immunofluorescence, and identified nuclear beta-catenin expression in IM cases only. The authors concluded that IM shares signaling pathways with Barrett esophagitis and similarly should be regarded premalignant.[8]

A retrospective study by Corica and colleagues reviewed the records of 53 patients with IM and identified no progression in long-term follow-up (median, 12 y). However, their study suffers from selection bias as most of their IM cases were diagnosed in cystectomy specimen from children with exstrophic bladder and therefore they were likely completely removed, decreasing the likelihood of malignant transformation.[9] Similarly, Smith and colleagues studied 136 cases of cystitis glandularis (n = 117) and IM (n = 19). In their cohort, pure IM had concurrent carcinoma in 37% of the cases [including adenocarcinoma in 2 patients (10%)]. Only 1 case with cystitis cystica glandularis (identified during follow-up for UC) developed recurrent UC 3 months later. None of their IM cases developed any bladder carcinoma. The authors concluded that although IM can be associated with a concurrent bladder carcinoma, it does not pose higher risk of developing malignancy than the normal population.[10]

All the published studies performed with the intention to identify an association between IM and cancer, are retrospective in nature and occur after removal of the area of IM. Once the metaplastic tissue is removed, the risk associated with that particular lesion disappears and makes it more difficult to prove the premalignant to malignant progression. In our study, we investigated the presence of specific genetic mutations in IM without dysplasia, proving that a subset of these lesions do harbor genetic alterations that are common in colon adenocarcinoma and, therefore, suggests that some of these lesions may represent precursors of malignancy.

In a recent study by Roy and colleagues, the authors studies 15 bladder adenocarcinomas for genomic alterations and copy-number changes in 51 cancer-related genes using next generation sequencing, and compared them with cohorts of high-grade UC and colorectal

TABLE 3. Demographic Findings of Colonic Adenoma Cases and Their Mutations

Case #	Sex	Age (y)	Location	Diagnosis	Mutation (MAF)
1	M	67	Right colon	TVA	*CTNNB1* S45P (57.1%) *KRAS* G13D (39.4%)
2	F	66	Right colon	TVA, HG dysplasia	*FBXW7* R465H (46.7%) *KRAS* A146T (40.6%)
3	F	66	Rectum	TVA	—
4	M	52	Transverse	Sessile TVA	*KRAS* G12S (59-74%) *FBXW7* R505C
5	F	59	Ileocecal valve	TVA	*APC* R1114 (35%) *APC* S1465fs (38.8%) *KRAS* G12D (43.5%)
6	M	83	Right colon	TVA, HG dysplasia	*APC* E1309 (43.5%) *KRAS* G12D (46.6%)
7	M	57	Sigmoid	TVA	N/A
8	M	50	Left colon	TA	N/A
9	F	59	Ileocecal valve	TVA	N/A
10	M	75	Cecum	TVA	*APC* E1309 (86%) *KRAS* G12D (43.5%)

F indicates female; HG, high grade; M, male; MAF, mutant allele frequency; N/A, not available; TA, tubular adenoma; TVA, tubulovillous adenoma.

adenocarcinomas. The authors identified at least 1 genomic alteration in 73% of their cases, the most frequent was in *TP53* (47%), followed by *PIK3CA* (p.E542K, p. C420R, and p.N1044K; 20%) and *KRAS* (p.G12A, p. G12D, and p.G13D; 20%). None of the bladder adenocarcinomas with mucinous features harbored any genomic alteration in the genes evaluated. In their study, mutation in *APC* and *CTNNB1* was restricted to tumors with enteric morphology.[11] Furthermore, mutation in *APC* was detected in 27% of their cohort, and all cases with *APC* mutation had also activation of *CTNNB1* (B-catenin).[11] More than 40% of colorectal adenocarcinomas harbor mutations in *APC*.[12] Although most cases of APC mutation lead to activation in *CTNNB1*, not all of the cases demonstrate defect in *CTNNB1*.[11] Similarly, in our study we identified a substitution—nonsense mutation *APC* p.R232 in 1 IM case without coexisting *CTNNB1* mutation. The *APC* p.R232 mutation has been reported in colonic adenocarcinomas.[13–15]

NRAS is an oncogene located on the short arm of chromosome 1. Most of the mutations are of missense substitution (97.9%), and have been mostly reported in malignant melanomas of the skin (15.2%). It has been seen in 3.7% of colon adenocarcinomas, and is associated with 1.3% of UC.[16] We found a novel *NRAS* p.G13D mutation in a case of bladder IM. This is the first report of this mutation. Also in our study, the high-grade papillary UC group revealed 20% mutation in *PIK3CA*, one with coexisting *TP53* and the other with coexisting *KRAS* mutations. Numerous studies have detected associations between mutation in *TP53* and high-grade UC, specifically between loss of function mutation in *TP53* and invasion into muscularis propria.[17–19]

The association between *PIK3CA* and UC has been previously reported. Sjodahl and colleagues studied 145 UC and report a significant frequency of mutation in *TP53* (36%), *PIK3CA* (17%), and *FGFR3* (65% in low grade, 22% in high grade) in UC, with highly significant association between combined activating mutations of *PIK3CA* and *FGFR3*. The authors also report 6 mutations in *HRAS*, 4 in *KRAS*, and none in *NRAS*. The authors did not include any IM or adenocarcinoma in their series.[18] Platt and colleagues identified 27% mutation in *PIK3CA* in 27% of UC in their series with the most frequent protein effect hotspot codons p.E542K and p.E545K. This study also did not include any IM or adenocarcinoma.[20] Janku et al[21] also report p.E545K mutation as the most frequent *PIK3CA* mutation in carcinomas (not limited to bladder); however, the authors did not identify any effect on outcome or prognosis. Our results are consistent with the findings by Platt and colleagues. In our series, 20% of UC revealed mutation in p.E575K. No *PIK3CA* mutation was identified in IM. We found mutation in *TP53* in 1 case of UC, and none of IM cases. In addition, 1 case showed concomitant mutations in *PIK3CA* and *KRAS* as previously shown by Platt et al.[20]

Our study is limited by low number of cases. We included only the gene mutation panel commonly used for colonic adenocarcinomas that did not include some genes that are more commonly mutated in UC. The colonic adenomas and UC showed findings previously reported to be characteristic of those lesions, supporting the robustness of the method used in this study. In addition, our samples were tested in CLIA-certified, CAP-accredited laboratories which technically validate their next generation sequencing-based diagnostic tests to accurately detect variants of clinical significance in routine formalin-fixed paraffin-embedded samples with low cross-contamination rates.

In summary, a small subset of cases with IM without dysplasia bear mutations in the genes commonly associated with colon adenocarcinoma, including colorectal cancer specific mutation in *APC* gene. This suggests that a small proportion of cases with IM, but without dysplasia could have a premalignant potential, possibly following the colorectal pathways of carcinogenesis.

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