Immunohistochemical Analysis of SMARCB1/INI-1 Expression in Collecting Duct Carcinoma

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OBJECTIVES Collecting duct carcinoma (CDC) is a rare and aggressive renal tumor with a tendency to involve the renal sinus. CDC displays variable morphologic features that can overlap with those of renal medullary carcinoma. The loss of *SMARCB1/INI1* tumor suppressor gene, initially found in pediatric malignant rhabdoid tumors of the central nervous system, kidneys, and soft tissues, was also recently described in renal medullary carcinoma. The current immunohistochemical study assessed SMARCB1/INI1 expression in a series of CDCs.

METHODS A total of 20 archival cases of CDC were used to construct a tissue microarray. Each tumor was spotted 3-7 times; benign tissue from the same specimen was also included when available. The immunoexpression of SMARCB1/INI1 was evaluated using BAF47, a monoclonal mouse antibody directed against the *SMARCB1/INI1* gene product. Nuclear staining was considered as indicative of SMARCB1/INI1 expression.

RESULTS The complete loss of SMARCB1/INI1 expression was observed in 3 of 20 cases of CDC. Another 3 cases revealed focal and weak intensity staining. The remaining tumors showed multifocal or diffuse SMARCB1/INI1 expression with variable staining intensity. No significant differences were found in the clinicopathologic and outcome features regarding SMARCB1/INI1 status.

CONCLUSIONS The complete loss of SMARCB1/INI1 immunoexpression was found in 15% of CDC. No differences were found between the SMARCB1/INI1 positive and negative cases regarding the clinicopathologic and outcome features. Our results suggest that some CDC cases might be associated with genetic alterations involving the *SMARCB1/INI1* gene. In addition, SMARCB1/INI1 immunoexpression seems to be of limited value in the differential diagnosis of CDC versus renal medullary carcinoma, although these results require additional validation. UROLOGY 78: 474.e1–474.e5, 2011. © 2011 Elsevier Inc.

Collecting duct carcinoma (CDC) is a rare and aggressive renal epithelial neoplasm that is thought to derive from the "principal cells" of the collecting duct system.[1] CDC typically affects males more often than females (2:1), with a mean age at occurrence of 55 years (range 13-83). The tumors typically present at an advanced stage, often with metastases. The diagnosis of CDC is based on the identification of a poorly circumscribed, infiltrative tumor composed of irregular channel-like spaces and papillary/tubular structures lined by high-grade, pleomorphic cells and surrounded by a prominent desmoplastic response.[2] To some extent, the diagnosis of CDC is made after the exclusion of other high-grade renal tumors that can be found involving the renal pelvis, such as invasive urothelial carcinoma and renal medullary carcinoma (RMC), which can depict overlapping morphologic and immunohistochemical features with the former. Distinguishing CDC from the aforementioned entities can be challenging, especially in needle biopsies, but the separation could be of clinical importance given the potential differences in the prognosis and treatment that these tumors exhibit.

More than 1 decade ago, *SMARCB1/INI1*, a highly evolutionarily conserved tumor suppressor gene component of a multiprotein complex involved in adenosine triphosphate-dependant chromatin remodeling, was found to be lost in malignant rhabdoid tumors of the childhood.[3,4] Since then, several studies have confirmed that

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most of these neoplasms contain bi-allelic inactivating mutations of the *SMARCB1/INI1* gene.[5] The loss of SMARCB1/INI1 expression has also been observed in most epithelioid sarcomas, one half of epithelioid malignant peripheral nerve sheath tumors, and RMCs[6-8]; nevertheless, experience with other types of renal cell carcinomas is limited.[7] We found no published reports evaluating the status of this tumor suppressor gene in CDC. The aims of the present study were to determine the SMARCB1/INI1, immunoexpression in a series of primary CDC cases to evaluate its usefulness in the differential diagnosis of RMC versus CDC, and to characterize the clinicopathologic and outcome features according to the SMARCB1/INI1 status.

MATERIAL AND METHODS

Patient Cohort

A total of 20 cases of CDC, diagnosed from 1990 to 2009, were identified from 3 participating institutions (Johns Hopkins Hospital, Baltimore, MD; Hacettepe University, Ankara, Turkey; and Universidade Estadual de Campinas School of Medicine, São Paulo, SP, Brazil). The diagnosis of CDC was confirmed in each case by 2 senior uropathologists on the study (G.J.N., J.I.E.) using the 2004 World Health Organization criteria for CDC.[1,2] None of the patients with CDC had evidence of a primary tumor elsewhere or a history of sickle cell disease/trait. Using a previously described procedure,[9] a tissue microarray was constructed. When available, benign negative controls for each case were also spotted. Follow-up was available for 14 patients and ranged from 2 to 151 months (mean 32).

Immunohistochemistry Analysis

Immunohistochemical analysis was performed on 5-μm-thick formalin-fixed paraffin-embedded tissue microarray sections using BAF47, a monoclonal mouse antibody directed against the *SMARCB1/INI* gene product (1:100, BD Transduction Laboratories, San Diego, CA). Staining was performed using an automated Bond-Leica biotin-free staining system according to the manufacturer's instructions (Leica Microsystems, Bannockburn, IL). The sections were deparaffinized and hydrated in a series of "dewax" solutions and alcohol. Heat-induced antigen retrieval was performed with a high pH antigen retrieval buffer (ER2). Incubation with primary antibody was followed by incubation with a secondary antibody and substrate. Finally, the sections were counterstained and coverslipped. Any nuclear staining, regardless of its intensity, was considered as indicative of SMARCB1/INI1 expression. The extent of positive staining was categorized as focal (positive in 10%-25% of cells), multifocal (25%-75%), or diffuse (>75%). Entrapped normal histologic structures and paired benign tissues were used as internal positive controls.

Statistical Analysis

The subset of cases with SMARCB1/INI loss was compared with those with maintained SMARCB1/INI1 immunoexpression to find significant differences in terms of patient demographics or outcome. A 2-tailed *P* value less than .05 was required for statistical significance. Analyses were done using the software STATA version 9.2 (StataCorp, College Station, TX).

RESULTS

Cohort Characteristics

The mean patient age at nephrectomy was 61 years (range 30-85). The male/female ratio was 2.4. The disease progression and disease-specific survival rate was 34% and 64%, respectively.

Immunohistochemical Findings

The patterns of SMARCB1/INI1 immunoexpression are depicted in Figure 1. The complete loss of SMARCB1/INI1 expression was observed in 3 (15%) of the 20 CDC cases. Another 3 cases revealed focal and weak staining. The remaining tumors showed multifocal to diffuse SMARCB1/INI1 expression, with a variable staining intensity. Immunoexpression of SMARCB1/INI1 was preserved in nontumoral control kidneys and entrapped benign renal tubules, even in those cases with SMARCB1/INI1 loss in the tumor cells.

SMARCB1/INI1 Status and Clinicopathologic Features

No significant differences were found regarding age, tumor size, pT stage, or vascular invasion between patients with and without SMARCB1/INI1 loss (Table 1). The loss of SMARCB1/INI1 in CDC did not predict disease progression or disease-specific survival in our cohort.

COMMENT

This is the first study evaluating the SMARCB1/INI1 status in CDC, a subtype of renal cell carcinoma accounting for <1% of all kidney cancers in adults and characterized by an aggressive clinical course. According to the most recent series, an appreciable proportion of patients present with disseminated disease, and the mortality rate is high.[1,2,7,10] These tumors are usually located in the central/medullary zone and exhibit ill-defined, nonencapsulated, infiltrative borders. Other high-grade tumors, including high-grade invasive urothelial carcinoma, papillary renal cell carcinoma type II, and RMC, can involve the same region of the kidney and enter the differential diagnosis. Microscopically, given its tubular/tubulopapillary morphology and intense stromal desmoplasia, CDC could be confused with RMC; the distinction can be particularly challenging in small tissue samples, such as those obtained by needle biopsies. The distinction of these 2 entities is important because of the potential differences in prognosis and therapy. Immunohistochemistry seems to be of limited usefulness because considerable overlap exists in the staining profiles of CDC and RMC.[2] Taking into account the recently described consistent loss of SMARCB1/INI1 expression in RMC,[6,7] the present study was designed to evaluate the status of this tumor suppressor gene in CDC. *SMARCB1/INI1*, located in chromosome 22q11.2, encodes for a 385-residue protein that is part of the SWI/SNF complex, a multiprotein complex involved in adenosine triphos-

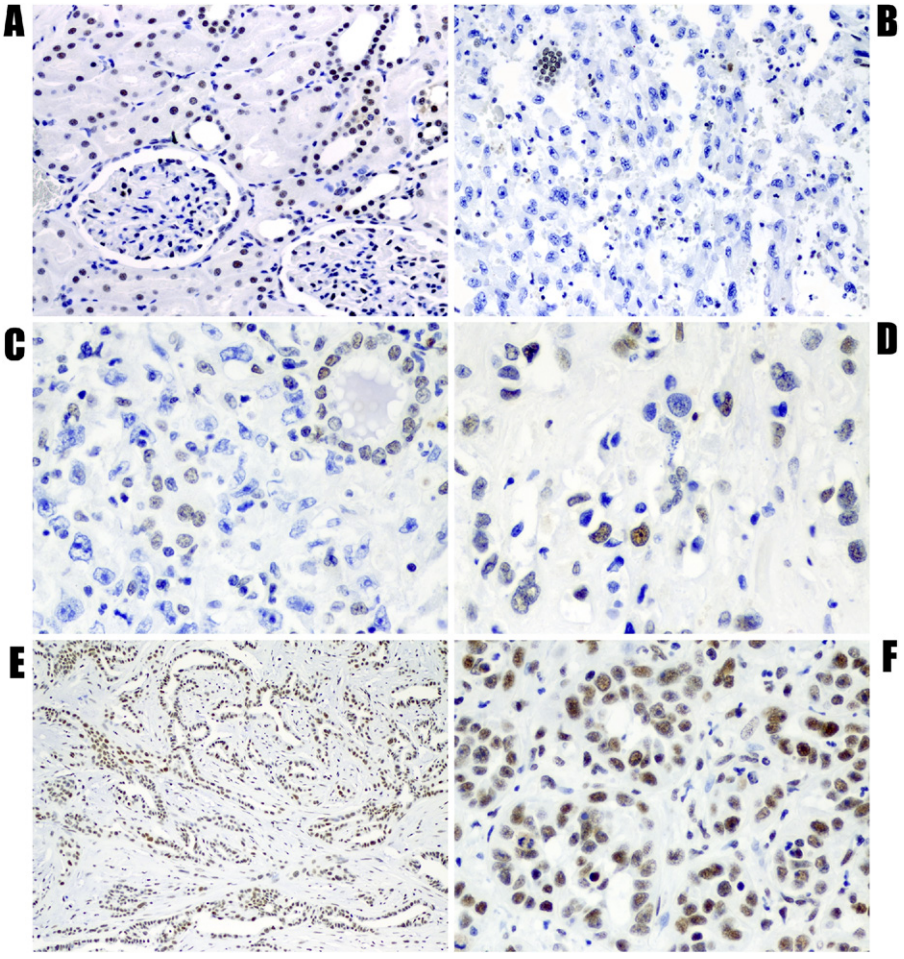

Figure 1. Patterns of SMARCB1/INI1 immunoexpression. **(A)** Benign renal tissue showing normal SMARCB1/INI1 expression in tubules and glomeruli. **(B)** CDC specimen without SMARCB1/INI1 immunoexpression. Entrapped renal tubules (upper left) and inflammatory cells are positive. **(C, D)** Scattered neoplastic cells with weak to moderate, focal, SMARCB1/INI1 immunoexpression. Benign entrapped tubule noted in upper right field of Fig. C. **(E, F)** CDC showing moderate to strong, diffuse, SMARCB1/INI1 immunoexpression.

Table 1. Clinicopathologic and outcome features of patients according to SMARCB1/INI1 status

	SMARCB1/INI Immunoexpression		
Variable	Negative	Positive	*P* Value
Mean age (y)	70.3	58.9	.36
Gender*			.99
Male	2 (67)	10 (71)	
Female	1 (33)	4 (29)	
Tumor size (cm)	5.8	7.1	.54
pT stage†			.41
pT1	1 (33)	1 (8)	
pT3	2 (67)	10 (77)	
pT4	0 (0)	2 (15)	
Vascular invasion‡	0/2 (0)	10/12 (83)	.07
Dead of disease	1/2 (50)	8/12 (67)	.99

* Datum not available for 3 patients.
† Disease stage not available for 4 patients.
‡ Vascular invasion not evaluable in 6 cases.

phate-dependent chromatin remodeling[11,12]; SWI/SNF have been identified in all eukaryotes, and SMARCB1/INI1 is a core subunit present in all variants of this complex. The loss of *SMARCB1/INI1* expression can be caused by inactivating deletions and coding sequence mutations of the corresponding 22q11.2 chromosomal band.[13] In animal models, homozygous inactivation of *SMARCB1/INI1* results in early embryonic death, but heterozygous individuals with germ line mutations are expected to be phenotypically normal.[5,14] However, around 20% of the heterozygous animals acquire malignant tumors, and the loss of heterozygosity results in the development of cancer within weeks in 100% of the population.[15-18] Convincing evidence has shown that those animal models are applicable to human tumors,[19] especially in light of the consistent loss of SMARCB1/INI1 immunoexpression in certain types of malignant neoplasms.[6,7] In this context, *SMARCB1/INI1* acts as a tumor suppressor gene, and it is the first member of an adenosine triphophatase chromatin remodeling complex to be implicated in the genesis of malignant tumors. The loss of *SMARCB1/INI1* causes tumor development mainly by disrupting the normal cell cycle and promoting cell cycle progression by way of downregulation of

p16^{INK4a} and upregulation of *E2Fs* and *cyclin D1*.[5,19,20] In addition, *SMARCB1/INI1* loss is associated with an increase in the activity of RhoA, a small guanosine triphosphatase involved in the formation of actin cytoskeleton stress fibers, increasing cell motility and conferring enhanced migratory potential to mutated cells.[21,22] This can explain the well-known clinical aggressiveness of tumors with the loss of SMARCB1/INI1 expression and their high metastatic rate.

Most of *SMARCB1/INI1* inactivation events have been found in rhabdoid tumors of the central nervous system, kidneys, and soft tissues, as well as medullary renal cell carcinoma.[5-7,13] Most of these renal tumors are seen in children, with only a small subset occurring during adulthood. The present series has expanded on the findings from previous studies by evaluating the SMARCB1/INI1 expression status in CDC. Considering that SMARCB1/INI1 immunoexpression was maintained in most, but not all, of our cases, its usefulness in the differential diagnosis of CDC versus RMC might be of limited value, if any. Thus, features other than the morphology, such as the patient's age and race and the presence of sickle cell disease/trait, should be taken into account for a proper diagnosis. Although our redundancy in tissue microarray spotting should ensure adequate representation of the tumor tissue, additional evaluation by immunohistochemistry and molecular approaches, including fluorescence in situ hybridization, single nucleotide polymorphism-based array analysis or another polymerase chain reaction-based technology,[13] would help confirm our present findings. Finally, given that invasive high-grade urothelial carcinomas involving the renal pelvis can also enter the differential diagnosis, we are evaluating SMARCB1/INI1 expression in such lesions. It is of interest to determine whether including the current marker in addition to our previously suggested PAX-8 and p63 panel[23] will be of any added value in resolving the differential diagnosis among high-grade renal sinus malignancies.

Our results indicate that some CDCs are associated with the loss of *SMARCB1/INI*. It would be of great interest to determine the underlying genetic mechanisms that lead to SMARCB1/INI1 loss in these cases and to compare such changes to those observed in other renal and extrarenal SMARCB1/INI1 negative tumors. The potential mechanisms to be investigated include the loss of expression caused by inactivating deletions and coding sequence mutations with or without loss of heterozygosity. Post-transcriptional alterations, including microRNA-related mechanisms, could also be explored. Given the recent interest in therapeutically targeting SMARCB1/INI1,[24] the determination of SMARCB1/INI1 status could be of additional interest in CDC.

CONCLUSIONS

We analyzed SMARCB1/INI1 status in a series of 20 CDC cases, and we observed the loss of expression in 15% of the cases. No differences were found between SMARCB1/INI1-positive and negative cases in regard to the clinicopathologic and outcome features. Our results suggest that some CDC cases might be associated with genetic alterations involving the *SMARCB1/INI1* gene. In addition, SMARCB1/INI1 immunoexpression seems to be of limited value in the differential diagnosis of CDC versus RMC, if our present findings are confirmed in additional cohorts.

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