

# Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior

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**Renal medullary carcinoma is a rare, well-recognized highly aggressive tumor of varied histopathology, which occurs in young patients with sickle cell trait or disease. Rhabdoid elements, occasionally seen in high-grade renal tumors including renal medullary carcinoma, possibly represent a pathologic marker of aggressive behavior. INI1 (hSNF5/SMARCB1/BAF47) is a highly conserved factor in the ATP-dependent chromatin-modifying complex. Loss of this factor in mice results in aggressive rhabdoid tumors or lymphomas. In humans, the loss of INI1 expression has been reported in pediatric renal rhabdoid tumors, central nervous system atypical teratoid/rhabdoid tumors and epithelioid sarcomas, a possible primary soft tissue rhabdoid tumor. This study compares five renal medullary carcinomas with 10 high-grade renal cell carcinomas (five with rhabdoid features), two urothelial carcinomas and two pediatric renal rhabdoid tumors. All five renal medullary carcinomas, irrespective of histopathology, showed complete loss of INI1 expression similar to that seen in pediatric renal rhabdoid tumors. In contrast, all renal cell carcinomas or urothelial carcinomas, including those with histological rhabdoid features, expressed INI1. Clinically, all five of the patients with renal medullary carcinoma and the two patients with rhabdoid tumors presented with extra-renal metastases at the time of diagnosis. This study demonstrates that renal medullary carcinoma and renal rhabdoid tumor share a common molecular/genetic alteration, which is closely linked to their aggressive biological behavior. However, the absence of INI1 expression is not necessarily predictive of rhabdoid histopathology but remains associated with aggressive behavior in renal medullary carcinoma.**

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Renal medullary carcinoma is a rare, highly aggressive primary renal tumor first described by Davis *et al*<sup>1</sup>, typically affecting young patients with sickle-cell trait or disease. This tumor predominantly arises in the renal medulla and may exhibit a variety of growth patterns including reticular, solid, tubular, trabecular, cribriform, sarcomatoid and micropapillary.<sup>1–7</sup> The tumor cells usually have large vesicular nuclei and prominent nucleoli with densely opaque eosinophilic cytoplasm. Eccentric nuclear displacement in some cases creates a rhabdoid appearance. The fatality approaches 100% within several weeks to months after diag-

nosis. An explanation for its aggressive behavior is unknown.

INI1 (hSNF5/SMARCB1/BAF47) is a highly conserved factor, from yeasts to humans, in an ATP-dependent chromatin-modifying complex-SWI/SNF.<sup>8</sup> Loss of this factor in transgenic mice results in aggressive rhabdoid tumors or lymphomas with 100% mortality.<sup>9,10</sup> In humans, loss of INI1 expression has been reported in pediatric renal and extrarenal malignant rhabdoid tumors,<sup>11,12</sup> atypical teratoid/rhabdoid tumors of the central nervous system<sup>12,13</sup> and epithelioid sarcomas, most likely a primary soft tissue rhabdoid sarcoma.<sup>14,15</sup> INI1 loss and rhabdoid histology are independently associated with aggressive tumor behavior. The recognized histologic spectrum of renal medullary carcinoma includes but is not restricted to rhabdoid morphology. The extent to which INI1 and rhabdoid-morphology may be linked, if at all, in those tumors may help to understand the biologic behavior.

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The present study investigates INI1 expression in renal medullary carcinomas and other high-grade renal tumors, including those with rhabdoid histologic features. The results show a loss of INI1 in all renal medullary carcinomas and pediatric renal rhabdoid tumors, but persistent INI1 expression in high-grade renal carcinomas including those with rhabdoid features. The results in this study demonstrate that loss of INI1 is a common feature of renal medullary carcinoma with or without rhabdoid morphology and may be linked to an underlying molecular mechanism, which may at least in part explain the extremely aggressive behavior.

## Materials and methods

Five renal medullary carcinomas were recovered from the archived surgical pathology files of the University of Chicago between 1994 and 2007. Table 1 summarizes the relevant clinical and pathological information. For comparison, 10 examples of high-grade renal cell carcinoma and two high-grade urothelial carcinomas of the renal pelvis were selected from recent surgical pathology accessions. Two cases of pediatric rhabdoid tumor were available, one from the archived surgical pathology files and one from the consultation files of one of the authors (JBT). All tumors had been thoroughly sampled for routine H & E light microscopy and a representative block from each case was selected for detailed further study.

INI1 immunohistochemistry was performed utilizing a mouse monoclonal antibody (BD Transduction Labs, San Diego, CA, USA) at 1:200

dilution. EZH2 (a factor in the polycomb chromatin-modifying complex) immunohistochemistry was performed using a polyclonal rabbit anti-human antibody (Zymed, San Francisco, CA, USA) at 1:1000 dilution. Slides were subjected to heat-induced epitope retrieval pretreatment in citrate buffer (pH 6.0) for 3 min, followed by cooling to room temperature. Sections were incubated with the primary antibodies for 2 h at room temperature. An additional panel of antibodies was used in each case: CD10 (Novocastra Laboratories Ltd, Newcastle Upon Tyne, United Kingdom, at 1:20 dilution), vimentin (DakoCytomation Inc., Carinteria, CA, USA at 1:100 dilution), epithelial membrane antigen (Cell Marque Corporation, Rocklin, CA, USA at 1:10 dilution), S100 (DakoCytomation Inc., at 1:100 dilution), cytokeratins AE1/AE3 (Signet Laboratories Ltd, Deham, MA, USA at 1:20), CAM5.2 (BD Biosciences, San Jose, CA, USA), CK7 and CK20 (DakoCytomation Inc., at 1:100 dilution). These immunohistochemical stains were carried out in routine fashion using a DAKO Autostainer.

## Results

A total of 19 cases were studied: five renal medullary carcinomas, two rhabdoid tumors of kidney, 10 renal cell carcinomas and two urothelial carcinomas. The salient clinical, histologic and immunohistochemical features are summarized in Table 1. All renal medullary carcinomas were young adults (three female, two male) with tumor sizes ranging from 5.5–10 cm. All patients were Black, all had sickle-cell trait and presented with extensive

**Table 1** Clinical and pathologic features of renal medullary carcinoma, rhabdoid tumors of kidney and other high-grade renal tumors

Patient	Age/sex	Diagnosis	Rhabdoid feature (%)	INI1	Metastasis
1 <sup>a</sup>	21 Years/F	RMC	Extensive (40)	Absent	Liver, lung
2 <sup>a</sup>	32 Years/M	RMC	< 5%	Absent	Liver, lung
3 <sup>a,b</sup>	28 Years/M	RMC	Focal (20)	Absent	Liver, lung
4 <sup>a</sup>	21 Years/F	RMC	Focal (10)	Absent	Liver, lung
5 <sup>a</sup>	22 Years/F	RMC	None (0)	Absent	Liver, lung
6	4 Years/F	RTK	Focal (30)	Absent	Lymph nodes
7	8 Years/M	RTK	Focal (10)	Absent	Brain
8	58 Years/F	(P.) RCC	Extensive (90)	Present	None
9	70 Years/M	(C.) RCC	Extensive (70)	Present	Lung, liver
10	58 Years/M	(C.) RCC	Focal (30)	Present	None
11	54 Years/F	(P.) RCC	Focal (20)	Present	None
12	79 Years/F	(Ch.) RCC	Focal (10)	Present	Lung
13	68 Years/M	(P.) RCC	Absent	Present	None
14	57 Years/M	(C.) RCC	Absent	Present	None
15	64 Years/M	(C.) RCC	Absent	Present	None
16	68 Years/M	(C.) RCC	Absent	Present	None
17	53 Years/M	(P.) RCC	Absent	Present	None
18	64 Years/F	UC (grade 4)	Absent	Present	None
19	74 Years/F	UC (grade 4)	Absent	Present	None

F, female; M, male; RMC, renal medullary carcinoma; RTK, rhabdoid tumor of kidney; RCC, renal cell carcinoma; UC, urothelial carcinoma of renal pelvis; C, clear cell; Ch, chromophobe; P, papillary.

<sup>a</sup>Sickle-cell trait.

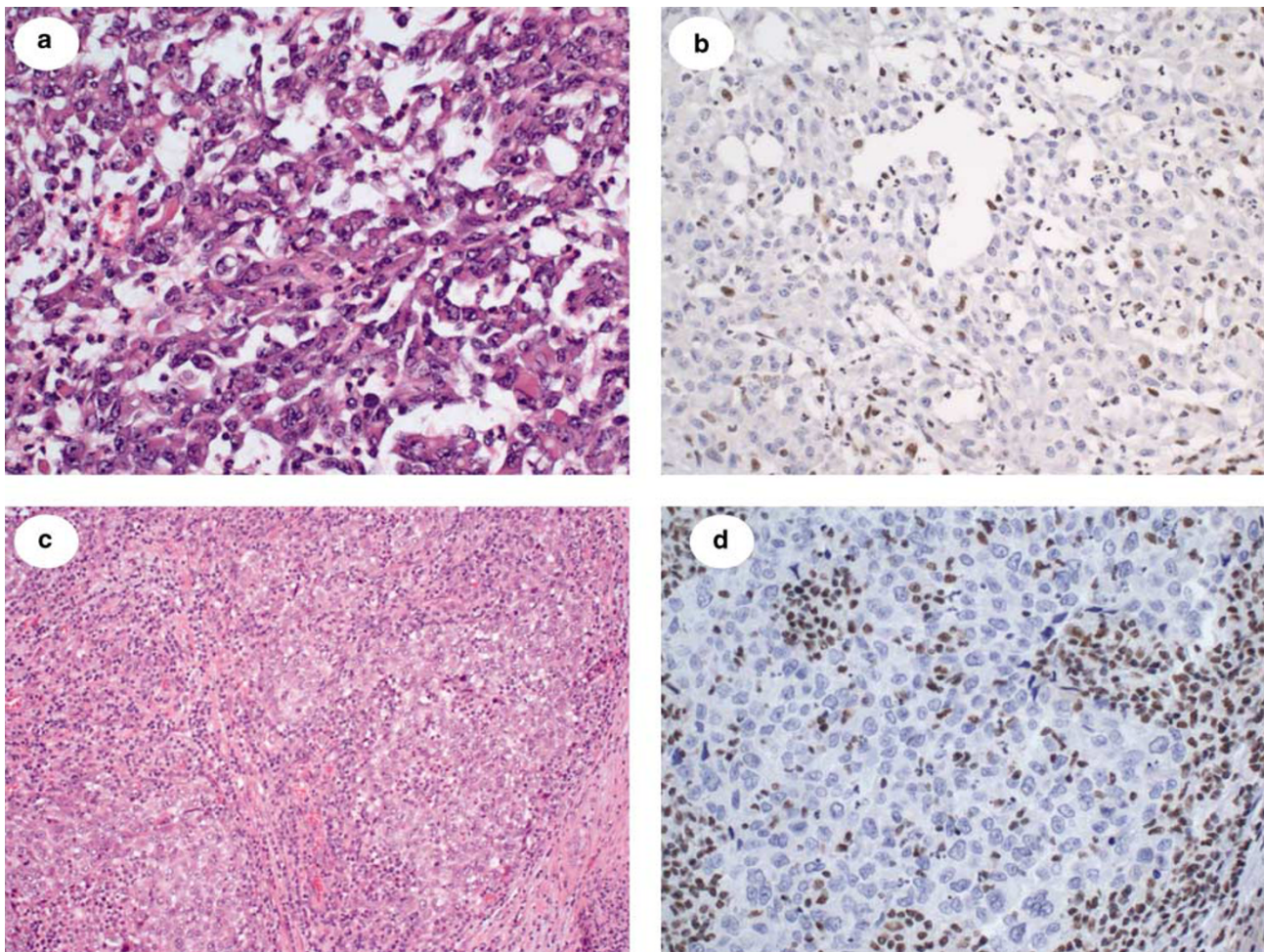
<sup>b</sup>Patient died 5 months post-diagnosis.

extrarenal metastases at the time of diagnosis. One of those patients died 5 months after diagnosis. In the comparison group of high-grade renal carcinomas, the sizes ranged from 4 to 11 cm. Five of the ten renal cell carcinomas had rhabdoid features histologically. Two had extrarenal metastases at the time of diagnosis. The two rhabdoid tumors of kidney were 11 and 8 cm.

The renal medullary carcinomas exhibited a broad spectrum of histologic features with rhabdoid features ranging from <5–50% of tumor cells in four cases. One had no rhabdoid features, although this tissue sample was a needle biopsy. Renal medullary carcinoma no. 1 (Figure 1a) showed a reticular pattern with rhabdoid cells: eccentrically placed vesicular nuclei, prominent nucleoli and dense, dark eosinophilic cytoplasm. Renal medullary carcinoma no. 2 had a diffuse pattern with less prominent rhabdoid cells (Figure 1c). The immunohistochemical studies are summarized in Table 2. INI1 immunohistochemical staining in these cases

(Figure 1b and d) was totally negative. Focal microcystic, trabecular and sarcomatoid patterns, in addition to rhabdoid areas, were all INI1 negative. Thus, there was a complete loss of INI1 in all five cases of renal medullary carcinoma, irrespective of the histopathologic features. Since INI1 is ubiquitously expressed in non-neoplastic nuclei, endothelial cells and lymphocytes within the tumor served as internal controls. Renal medullary carcinomas were also positive for EZH2, a factor in the polycomb chromatin-modifying complex; vimentin, epithelial membrane antigen, cytokeratin AE1/AE3 and CAM5.2 were also positive as shown in Table 2.

Histologic examination of the high-grade renal cell carcinomas and urothelial carcinomas of the renal pelvis demonstrated rhabdoid morphology in five cases of renal cell carcinoma. In Case 8 (Figure 2a), the tumor cells having predominant rhabdoid features (Figure 2a) showed strong nuclear reactivity to INI1 (Figure 2b). The sarcomatoid



**Figure 1** Morphological features and INI1 immunohistochemistry of renal medullary carcinomas. (a) Hematoxylin and Eosin stain of Case 1 demonstrating a reticular histologic pattern with rhabdoid type cells. (b) INI1 immunohistochemical stain of Case 1 showing absent nuclear staining. (c) Hematoxylin and Eosin stain of Case 2 with a solid growth pattern. (d) INI1 immunohistochemical stain of Case 2; nuclear staining absent. Positive nuclear staining is seen in background lymphocytes.

features of Case 12 (Figure 2c) also demonstrated strong INI1 reactivity (Figure 2d). Reactions for the remaining immunohistochemical markers were similar to the renal medullary carcinoma cases.

Two primary rhabdoid tumors of the kidney showed typical histologic features (Figure 3). There

**Table 2** Immunohistochemical summary

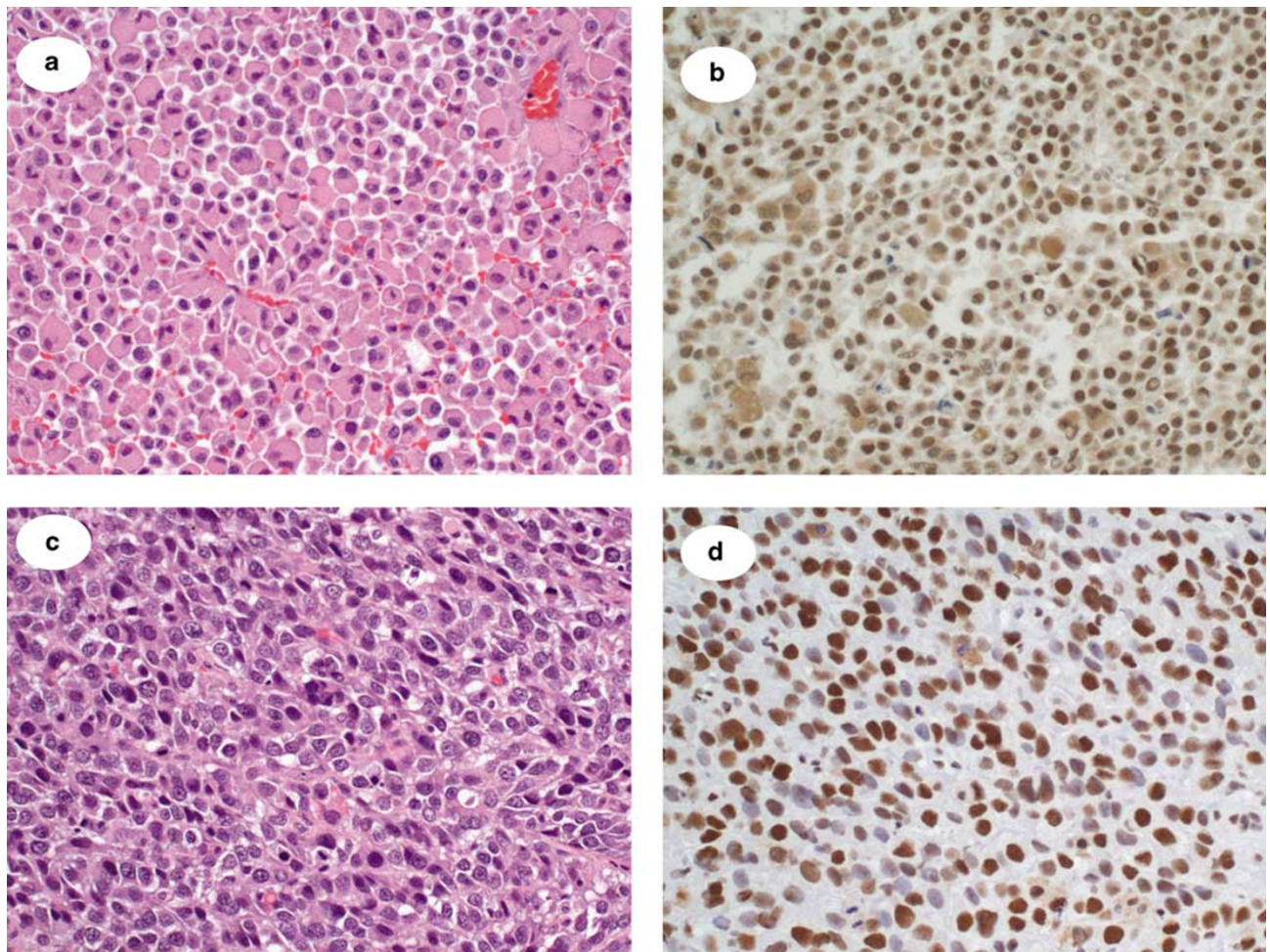
	INI1	EZH2	Vimentin	EMA	CK (AE1/AE3, CAM5.2)
Renal medullary carcinoma (5)	–	+	+	+	+
Rhabdoid tumor of kidney (2)	–	+	+	+	±
Renal cell carcinoma (10)	+	+	+	+	+
Urothelial carcinoma of renal pelvis (2)	+	+	ND	+	+

ND, not done.

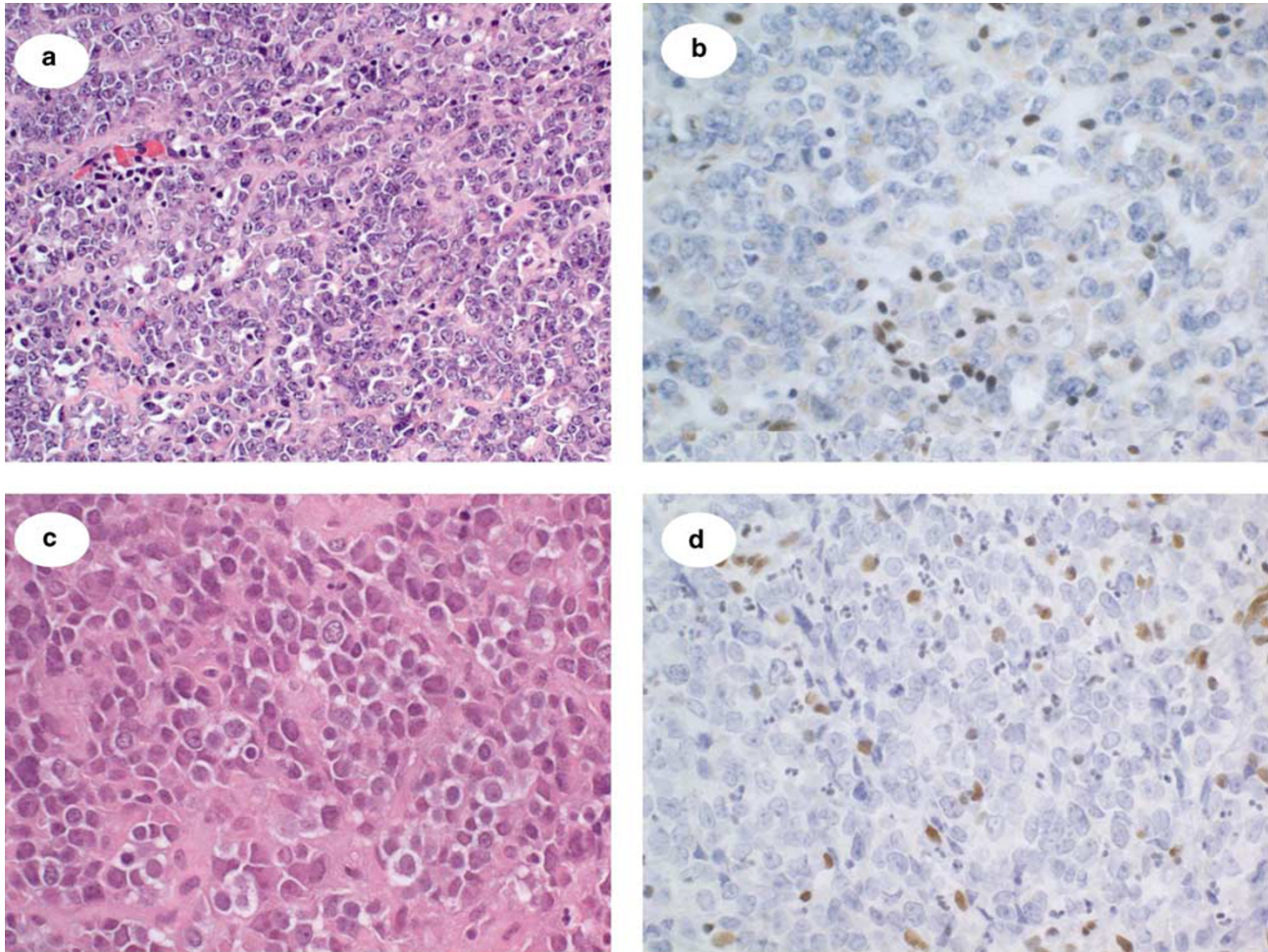
was a complete loss of INI1 staining in both cases. The remaining antibody panel was positive as found in the renal cell carcinoma and renal medullary carcinoma groups.

## Discussion

The rhabdoid phenotype in tumors of the kidney is evidence of potentially aggressive behavior. This includes tumors in the pediatric group and less commonly in adults.<sup>1–7,16,17</sup> Prominent rhabdoid features have also been identified and are characteristic of epithelioid sarcoma and atypical/teratoid tumors of the central nervous system. The rare renal medullary carcinoma seems restricted to patients with sickle-cell disease or trait,<sup>1–7</sup> and despite varied histologic growth patterns also manifests variably prominent rhabdoid cytology. The possibility of a genetic connection between a rhabdoid cytologic phenotype and the aggressive biologic behavior of tumors, which exhibit this feature has not yet been elucidated.



**Figure 2** Morphological features and INI1 immunohistochemistry of renal cell carcinomas with rhabdoid cytologic features. (a) Hematoxylin and Eosin stain of Case 8 with prominent rhabdoid features. (b) INI1 immunohistochemical stain of Case 8 showing positive nuclear reactions. (c) Hematoxylin and Eosin stain of Case 12 with solid growth and focal rhabdoid features. (d) INI1 immunohistochemical stain of Case 12 with strongly positive nuclear reactions.



**Figure 3** Morphological features and INI1 immunohistochemistry of rhabdoid tumors of kidney. (a) Hematoxylin and Eosin stain of Case 6. (b) INI1 immunohistochemical stain of Case 6 demonstrating a diffuse negative nuclear reaction. (c) Hematoxylin and Eosin stain of Case 7. (d) INI1 negative immunohistochemical stain of Case 7. Positive nuclear staining is seen in background lymphocytes.

INI1 protein is a member of the SWI/SNF multi-protein complex, which is involved in chromatin modification in an ATP-dependent manner.<sup>8</sup> Loss of this factor in mice results in aggressive rhabdoid tumors or lymphomas with 100% mortality.<sup>9,10</sup> The function of this protein is incompletely understood. Previous studies of pediatric rhabdoid tumors have shown uniform absence of immunoreactivity to INI1 protein while previous studies of rhabdoid elements in renal cell carcinomas have shown positive reactions.<sup>11–13</sup> It is possible that the absence of INI1 staining in primary rhabdoid tumors of the kidney and renal medullary carcinomas indicates a common biallelic inactivation of the INI1/hSNF5 tumor suppressor gene of chromosome 22q11.2. Recently, Sigauke *et al*<sup>12</sup> also reported the absence of INI1 immunoreactivity in one renal medullary carcinoma case of a series of extrarenal and renal rhabdoid tumors.

In the present series, in addition to the loss of INI1 expression in two pediatric rhabdoid tumors of kidney, there was a complete absence of INI1 in all five renal medullary carcinoma cases exhibiting

varying histologic patterns, including rhabdoid features. All high-grade renal cell carcinomas and urothelial carcinomas of renal pelvis in the present study, even those with rhabdoid features, retained strong INI1 immunohistochemical reactivity. This study did not pursue further identifying INI1/hSNF5 alterations using FISH, Reverse Transcriptase-PCR, DNA sequencing or other molecular methods. Previous studies have shown that up to 20–30% of renal and extra-renal rhabdoid tumors with loss of immunoreactivity to INI1 protein do not show alterations at the DNA level.<sup>11,12,18,19</sup> If INI1 has a diagnostic significance, it is, therefore, at the level of protein expression by INI1 immunohistochemistry in support of the morphologic diagnosis of malignant rhabdoid tumor. In the present series, the relation between INI1 immunoreactivity and potential upstream molecular alterations, while interesting, does not add diagnostic relevance and is beyond the scope of this study.

The results of this study suggest that rhabdoid cytologic features, while indicative of biologic aggressiveness in the neoplasms in which it has

been described,<sup>11–16</sup> may represent a phenotypic change or series of changes for which loss of INI1 expression may be one factor. A recent report showed that INI1-deficient tumors and rhabdoid tumors are convergent, but not fully overlapping entities.<sup>20</sup> Loss of INI1 is not restricted to pediatric renal rhabdoid tumors, as the present study has shown, since INI1 expression is lost in renal medullary carcinomas as well. Renal medullary carcinoma, an admittedly rare primary renal tumor, seems to represent a specific clinicopathologic entity, occurring thus far only in patients with sickle-cell trait or disease. However, INI1-loss tumors have been identified in a spectrum of organ systems.<sup>11–15</sup> While those tumors are histologically unrelated, the loss of this protein most likely identifies an underlying molecular aberration accounting for the aggressive clinical behavior.

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### Disclosure/conflict of interest

The authors have no conflict of interest to disclose.

### References

- 1 Davis Jr CJ, Mostofi FK, Sesterhenn IA. Renal medullary carcinoma: the seventh sickle cell nephropathy. *Am J Surg Pathol* 1995;19:1–11.
- 2 Coogan CL, McKiel Jr CF, Flanagan MJ, *et al*. Renal medullary carcinoma in patients with sickle cell trait. *Urology* 1998;51:1049–1050.
- 3 Swartz MA, Karth J, Schneider DT, *et al*. Renal medullary carcinoma: clinical, pathologic, immunohistochemical, and genetic analysis with pathogenetic implications. *Urology* 2002;60:1083–1089.
- 4 Assad L, Resetkova E, Oliveira VL, *et al*. Cytologic features of renal medullary carcinoma. *Cancer* 2005;105:28–34.
- 5 Watanabe IC, Billis A, Guimaraes MS, *et al*. Renal medullary carcinoma: report of seven cases from Brazil. *Mod Pathol* 2007;20:914–920.
- 6 Figenshau RS, Basler JW, Ritter JH, *et al*. Renal medullary carcinoma. *J Urol* 1998;159:711–713.

- 7 Dimashkieh H, Choe J, Mutema G. Renal medullary carcinoma: a report of 2 cases and review of the literature. *Arch Pathol Lab Med* 2003;127:135–138.
- 8 Schnitzler GR, Sif S, Kingston RE. A model for chromatin remodeling by the SWI/SNF family. *Cold Spring Harb Symp Quant Biol* 1998;63:535–543.
- 9 Roberts CW, Leroux MM, Fleming MD, *et al*. Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene *Snf5*. *Cancer Cell* 2002;2:415–425.
- 10 Roberts CW, Orkin SH. The SWI/SNF complex: chromatin and cancer. *Nat Rev Cancer* 2004;4:133–142.
- 11 Hoot AC, Russo P, Judkins AR, *et al*. Immunohistochemical analysis of hSNF5/INI1 distinguishes renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. *Am J Surg Pathol* 2004;28:1485–1491.
- 12 Sigauke E, Rakheja D, Maddox DL, *et al*. Absence of expression of SMARCB1/INI1 in malignant rhabdoid tumors of the central nervous system, kidneys and soft tissue: an immunohistochemical study with implications for diagnosis. *Mod Pathol* 2006;19:717–725.
- 13 Haberler C, Laggner U, Slavc I, *et al*. Immunohistochemical analysis of INI1 protein in malignant pediatric CNS tumors: lack of INI1 in atypical teratoid/rhabdoid tumors and in a fraction of primitive neuroectodermal tumors without rhabdoid phenotype. *Am J Surg Pathol* 2006;30:1462–1468.
- 14 Guillou L, Wadden C, Coindre JM, *et al*. ‘Proximal-type’ epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features. Clinicopathologic, immunohistochemical, and ultrastructural study of a series. *Am J Surg Pathol* 1997;21:130–146.
- 15 Haberler C, Laggner U, Slavc I, *et al*. SMARCB1/INI1 tumor suppressor gene is frequently inactivated in epithelioid sarcomas. *Cancer Res* 2005;65:4012–4019.
- 16 Gokden N, Nappi O, Swanson PE, *et al*. Renal cell carcinoma with rhabdoid features. *Am J Surg Pathol* 2000;24:1329–1338.
- 17 Fanburg-Smith JC, Hengge M, Hengge UR, *et al*. Extrarenal rhabdoid tumors of soft tissue: a clinicopathologic and immunohistochemical study of 18 cases. *Ann Diagn Pathol* 1998;2:351–362.
- 18 Biegel JA, Zhou JY, Rorke LB, *et al*. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. *Cancer Res* 1999;59:74–79.
- 19 Biegel JA, Kalpana G, Knudsen ES, *et al*. The role of INI1 and SWI/SNF complex in the development of rhabdoid tumors: meeting summary from the Workshop on Childhood Atypical Teratoid/Rhabdoid Tumors. *Cancer Res* 2002;62:323–328.
- 20 Bourdeaut F, Fréneaux P, Thuille B, *et al*. hSNF5/INI1-deficient tumours and rhabdoid tumours are convergent but not fully overlapping entities. *J Pathol* 2007;211:323–330.