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Anaplastic sarcomas of the kidney are characterized by *DICER1* mutations

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Anaplastic sarcoma of the kidney is a rare tumor (≤25 reported cases) characterized by the presence of cysts, and solid areas composed of bundles of undifferentiated spindle cells, showing marked cellular anaplasia (usually accompanied by TP53 overexpression). These tumors often feature prominent areas of cartilage or chondroid material. Germline mutations in *DICER1*, encoding the microRNA (miRNA) processor DICER1, cause an eponymous syndrome. Recent reports suggest that anaplastic sarcoma of the kidney should be included in DICER1 syndrome as germline *DICER1* mutations are associated with the occurrence of such tumors. Therefore, we sought to determine the following: (1) what proportion of anaplastic sarcoma of the kidney have *DICER1* mutations; (2) whether the identified mutations affect both alleles of *DICER1* (ie, are biallelic); (3) whether somatic missense mutations in the DICER1 RNase IIIb domain impact miRNA generation; and (4) whether *TP53* alteration always occurs in these tumors. *DICER1* mutations were evaluated by Sanger sequencing and next-generation sequencing in nine tumor/normal pairs. Impact of *DICER1* mutations on miRNA generation was evaluated via an *in vitro* DICER1 cleavage assay. TP53 status was assessed by immunohistochemistry and next-generation sequencing. Eight of the nine cases had at least one RNase IIIb *DICER1* mutation that impacted the generation of miRNAs. There were six tumors with truncating *DICER1* mutations and in four of them, the mutation found in the tumor was also detected in adjacent normal tissue, and therefore was likely to be either mosaic or germline in origin. Analysis of mutation phase revealed that two of three tumors had biallelic *DICER1* mutations. Six of nine anaplastic sarcomas of the kidney had aberrant TP53 immunohistochemisty with damaging *TP53* mutations identified in three cases. Taken together, these data suggest that the great majority of anaplastic sarcomas of the kidney have *DICER1* mutations and confirm that these tumors are part of the DICER1 syndrome.
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Anaplastic sarcoma of the kidney was first described as a novel pediatric renal neoplasm in 2007.[1] Previous to this formal description of anaplastic sarcoma of the kidney, some of these lesions could have been included in renal tumors broadly characterized as embryonal sarcomas of the kidney.[2,3] In a series of 25 such tumors,[2] 3 possessed anaplasia, so perhaps these tumors could be considered to be unrecognized anaplastic sarcomas of the kidney. Others may have been primary renal synovial sarcomas.[4] The one almost certain anaplastic sarcoma of the kidney identified before the paper of

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Vujanic *et al* in 2007 was published by Faria and Zerbini[5] as a dedifferentiated cystic nephroma. In this case, the renal tumor occurred in a 26-month-old girl. It was largely cystic, but had a solid anaplastic region with cartilaginous and rhabdomyoblastic differentiation.[5] In view of subsequent findings, it is perhaps surprising that neither germline nor somatic *DICER1* mutations were identified in this person or the tumor 18 years later.[6]

Anaplastic sarcoma of the kidney presents as a large renal mass, and its major gross and histologic features include the presence of cysts, marked anaplasia in the spindle cell component, and areas of benign or malignant cartilage or chondroid differentiation.[1] There is female predominance. In 2007, there were no definitive genetic mutations or molecular markers linked to these tumors. These tumors remain amongst the rarest renal neoplasms, with no more than 25 cases reported to date.

Recent studies have reported that a few isolated cases of ASK harbor mutations in *DICER1*,[6–9] making a case that anaplastic sarcomas of the kidney should be included with the other lesions of the pleiotropic tumor predisposition syndrome known as DICER1 syndrome (OMIM 606241). DICER1 syndrome tumors include pleuropulmonary blastoma,[10] cystic nephroma,[11] and many other rare tumor entities, mainly occurring in the pediatric and adolescent age range.[12] DICER1 is an endoribonuclease central to generating microRNAs (miRNAs), small RNA molecules that downregulate the expression of ~30% of protein-coding genes.[12] DICER1 utilizes its RNase IIIa and IIIb endonuclease domains to cleave precursor (pre)-miRNA stemloops, thus releasing the mature single-strand miRNA. Of note, mature miRNAs can be coded within either the 5′ (5p) or 3′ (3p) arms of pre-miRNA stemloops.[12] DICER1-related tumors usually possess two *DICER1* mutations: one predicted to result in a truncated protein and the other a missense mutation at specific residues within exons encoding the RNase IIIb domain of the DICER1 protein.[12] Previous studies of DICER1 syndrome-related tumors have shown that when two *DICER1* mutations are present in the tumor, one mutation is present on one of the two *DICER1* alleles and the other is present on the alternate allele[13–20] (ie, the mutations are said to be *in trans*, or are biallelic). In contrast, if both the mutations occur on one allele, then they are *in cis*, or monoallelic.

Pleuropulmonary blastomas with a germline *DICER1* mutation can occur with and without deleterious mutation in *TP53*.[13,18] Pleuropulmonary blastoma and anaplastic sarcoma of the kidney could be analogous tumors occurring in different organs; it has been suggested that the stages of DICER1-dependent pleuropulmonary blastomas could be reminiscent of the possible progression of DICER1-dependent cystic nephroma to anaplastic sarcoma of the kidney[6] but it is unknown whether all anaplastic sarcomas of the kidney arise from pre-existing cystic nephromas,[1,5–9,21–23] partly because of the rarity of anaplastic sarcomas of the kidney but also because the surgical removal of cystic nephromas will prevent the observation of development of anaplastic sarcoma of the kidney from cystic nephroma. Nevertheless, cystic nephromas can pre-exist in regions of the kidney where anaplastic sarcoma of the kidney have later been observed.[6–8] In this report, we sought to determine in nine anaplastic sarcomas of the kidney—(1) the frequency of *DICER1* mutations; (2) whether these mutations are biallelic; (3) whether the identified *DICER1* mutations occurring in the RNase IIIb domain affect pre-miRNA processing; and (4) does aberrant TP53 status always accompany *DICER1* mutations in anaplastic sarcomas of the kidney.

Materials and methods

Sample Acquisition and Histopathological Description of the Anaplastic Sarcomas of the Kidney

Nine anaplastic sarcomas of the kidney and matched normal kidney formalin-fixed paraffin-embedded specimens samples were obtained. Cases 1, 2, and 6 were described in the original description of anaplastic sarcoma of the kidney[1] and case 8 was presented by current author Watanabe,[23] however neither study investigated *DICER1* mutation status. Cases 7 and 9 have been reported by our group (refs 7 and 8, respectively) as DICER1-related anaplastic sarcomas of the kidney. The diagnosis in case 5 was more equivocal than for the other eight cases, and it was considered that it could represent an anaplastic Wilms tumor (the original diagnosis, Table 1).

The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine of McGill University, Montreal, QC, Canada (numbers A05-M60-14B, A08-M61-09B, and A12-M117-11A). Participants were recruited to the study in compliance with the second edition of the Canadian Tri-Council Policy Statement of Ethical Conduct of Research involving Humans and, where indicated because of young participant age, eligible relatives signed a consent form in accordance with the above-mentioned IRB protocols.

The anaplastic sarcomas of the kidney showed characteristic gross and histological features, including the presence of cysts (in the majority of cases) and solid areas composed of undifferentiated spindle cells with marked anaplastic changes, and prominent areas of benign or malignant cartilage or chondroid differentiation.[1] Other, less common features include blastema-like areas, foci of rhabdomyoblastic differentiation, and small islands of osteoid.[1]

Screening DICER1 Mutations in Anaplastic Sarcoma of the Kidney

DNA was extracted from formalin-fixed paraffin-embedded samples and *DICER1* RNase IIIa/b domain Sanger sequencing was performed as previously

Table 1 Clinicopathological features and follow-up

ASK	Age/sex	Side	Notable gross pathological finding	Initial pathological diagnosis	Stage	Treatment	Follow-up	Outcome
1	36 mo/F	L	$D = 15$ cm	Anaplastic Wilms tumor	I	N+CHT	No recurrence	NED 14 yrs
2	120 mo/M	L	$D = 17$ cm; $W = 1210$ g; nodular mass protruding into renal pelvis	Sarcomatoid variant of renal cell carcinoma	I	N+CHT	No recurrence	NED 8 yrs
3	139 mo/M	R	$D = 8$ cm; $W = 296$ g solid; intra-pelvic growth	Anaplastic Wilms tumor	II	CHT+N+CHT	Unknown	Unknown
4	106 mo/F	L	$D = 5$ cm; $W = 224$ g; 2/3 cystic	Anaplastic Wilms tumor *vs* ASK	I	N+CHT	Unknown	Unknown
5	18 mo/M	R	$D = 28$ cm; $W = 3400$ g; solid/cystic mass	Anaplastic Wilms tumor	III	CHT+N+CHT	Unknown	Unknown
6	18 mo/M	R	$D = 10$ cm; $W = 670$ g; solid/cystic mass protruding into renal pelvis	Anaplastic Wilms tumor	I	N+CHT	No recurrence	NED 13 yrs
7	10 mo/F or 105 mo/F	L	ASK $D = 12.9$ cm	Renal cysts identified at 10 mo Anaplastic sarcoma of the kidney at 105 mo	III	R+CHT	No recurrence	NED 12 yrs
8	156 mo/M	R	$D = 16$ cm; $W = 666$ g	Anaplastic Wilms tumor	III	CHT+R	Recurrence of 3 cm mass in right upper retroperitoneum	DOD 5 mo
9	7 mo/F	L	$D = 18$ cm; $W = 1620$ g; cystic mass	Anaplastic sarcoma of the kidney	I	N	No recurrence	NED 2 yrs

Abbreviations: CHT, chemotherapy; CCSK, clear cell sarcoma of kidney; *D*, diameter; DOD, died of disease; F, female; M, male; N, nephrectomy; NED, no evidence of disease; RT, radiation therapy; *W*, weight.
Details regarding treatment of cases 1–6 are discussed in ref. 1 and of case 8 in ref. 23. DICER1 results were previously reported for cases 7 and 9 and details concerning their features and follow-up for case 7 are in ref. 7, and for case 9 in ref. 8.

described.[20] The ability of missense mutations to cause exon skipping was assessed as reported by our group.[20] Additional fresh frozen tissue was available for three samples. In these cases, full *DICER1* Sanger sequencing was performed and the phase of mutations was determined via cloning[20] (Supplementary Data). Phase refers to whether two mutations are on the same copy of the gene (*in cis*) or whether there is one mutation on each chromosomal copy of *DICER1* (*in trans* or biallelic). In addition, DNA extracted from formalin-fixed paraffin-embedded tissues (normal and tumor) was subjected to a custom-designed standard HaloPlex panel containing 11 genes (full gene or targeted regions of *DICER1*, *SMARCA4*, *SMARCB2*, *CTNNB1*, *APC*, *BRAF*, and *PTCH1* as well as the exonic regions of *DROSHA*, *FGF3*, *FGFR1*, and *TP53*) according to a modified version of a previously published protocol.[24] This gene panel was designed by our group to use for several projects; however, only *DICER1* and *TP53* were of interest for the current study. The subsequent deep sequencing was performed at the McGill University and Genome Quebec Innovation Center. Sequence analysis was carried out using a modification of established protocols (Supplementary Materials).

In Vitro Cleavage Assay

HEK 293 cells were transduced to stably express FLAG-tagged versions of the somatically acquired *DICER1* RNase IIIb mutations. The ability of the FLAG-immunoprecipitated mutant proteins to cleave internally radiolabeled *in vitro*-transcribed pre-miR122 was evaluated in a time course by autoradiography of RNA products resolved by denaturing urea polyacrylamide electrophoresis. The details of this assay have previously been described.[19]

Immunohistochemistry

Immunohistochemistry for cases 1–8 was performed at the Segal Cancer Centre Research Pathology Facility (Jewish General Hospital). Tissue samples were cut at 4 μm, placed on SuperFrost/Plus slides (Fisher), and dried overnight at 37 °C, before immunohistochemical processing. The slides were then loaded onto the Discovery XT Autostainer (Ventana Medical System). All solutions used for automated immunohistochemisty were from Ventana Medical System unless otherwise specified. Slides underwent de-paraffinization and heat-induced epitope retrieval (CC1 pre-diluted solution Ref: 950–124, standard protocol). Immunostaining for TP53 was performed in an automated manner using a heat protocol. Briefly, pre-diluted mouse monoclonal anti-TP53 antibody (Clone Bp53-11, Ventana Medical Systems) was applied for 32 min at 37 °C then followed by the appropriate detection kit (OmniMap anti-Mouse-HRP, Ref: 760–4310) for 8 min, followed by ChromoMap-DAB (Ref: 760–159). A negative control was performed by omission of the

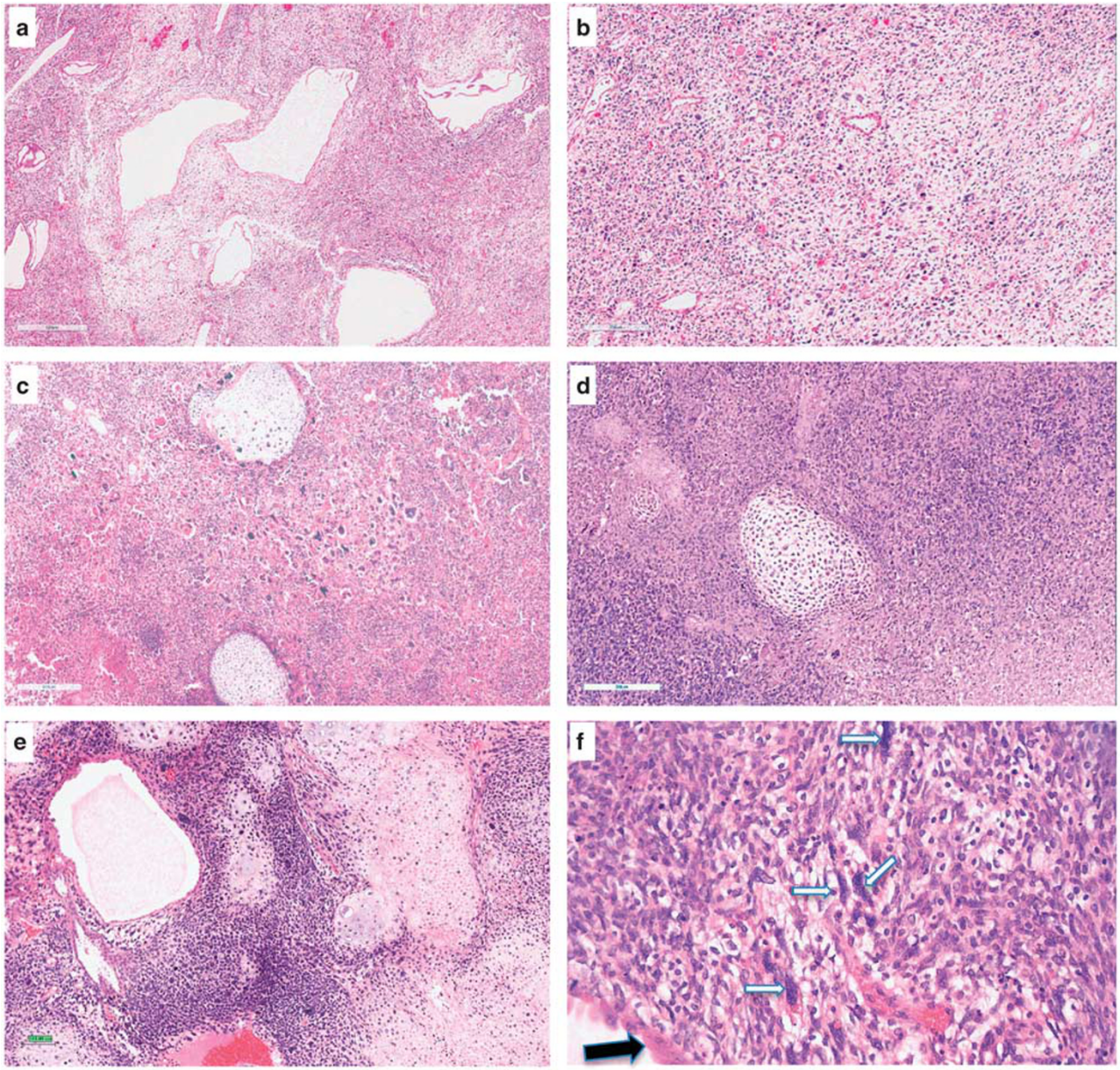


Figure 1 Anaplastic sarcoma of the kidney. (**a**) Small cysts surrounded by the stromal component, showing marked pleomorphism in (**b**); (**c**) anaplastic changes in the stroma and two islands of cartilage, one (upper) showing anaplastic changes; (**d**) an island of cartilage in the blastema-like area; (**e**) Case 8 H&E stained section at ×10 shows multifocal islands of cartilage and small cysts in the blastema-like area with necrosis. (**f**) Case 9 H&E stained section at × 200 shows a predominantly round and ovoid cell population expanding the wall of a cystic structure, which is lined by hobnail epithelium (black arrow). Marked nuclear pleomorphism features in this anaplastic sarcoma (white arrows). (**g–j**) Case 5. (**g**) Areas showing prominent rhabdomyoblastic differentiation. (**h**) A similar area with rhabdomyoblastic differentiation; (**i**) a spindle cell component of the tumor with some pleomorphic cells; (**j**) a mixture of areas with spindle cell and rhabdomyoblastic differentiation.

primary antibody. Slides were counterstained with hematoxylin. Sections were scanned using the Aperio A Turbo and analyzed by Drs G Vujanic and A Spatz. Case 9 was stained with Cell Marque antibody P53 (DO7) at 1:300 dilution.

Results

Histological characteristics of some of the anaplastic sarcomas of the kidney are described in the legend of Figure 1. Clinicopathological features and follow-up are presented in Table 1. Somatically acquired *DICER1* RNase IIIb mutations were identified in eight of the nine anaplastic sarcomas of the kidney studied (Figure 2a). These were determined to be somatically acquired as the mutation is present in the tumor DNA but absent in the matched normal tissue. Seven manifested as missense mutations (cases 2: c.5437G>A [p.E1813K]; 3: c.5113G>A [p.E1705K]; 4 and 7: c.5425G>A [p.G1809R]; 6 and

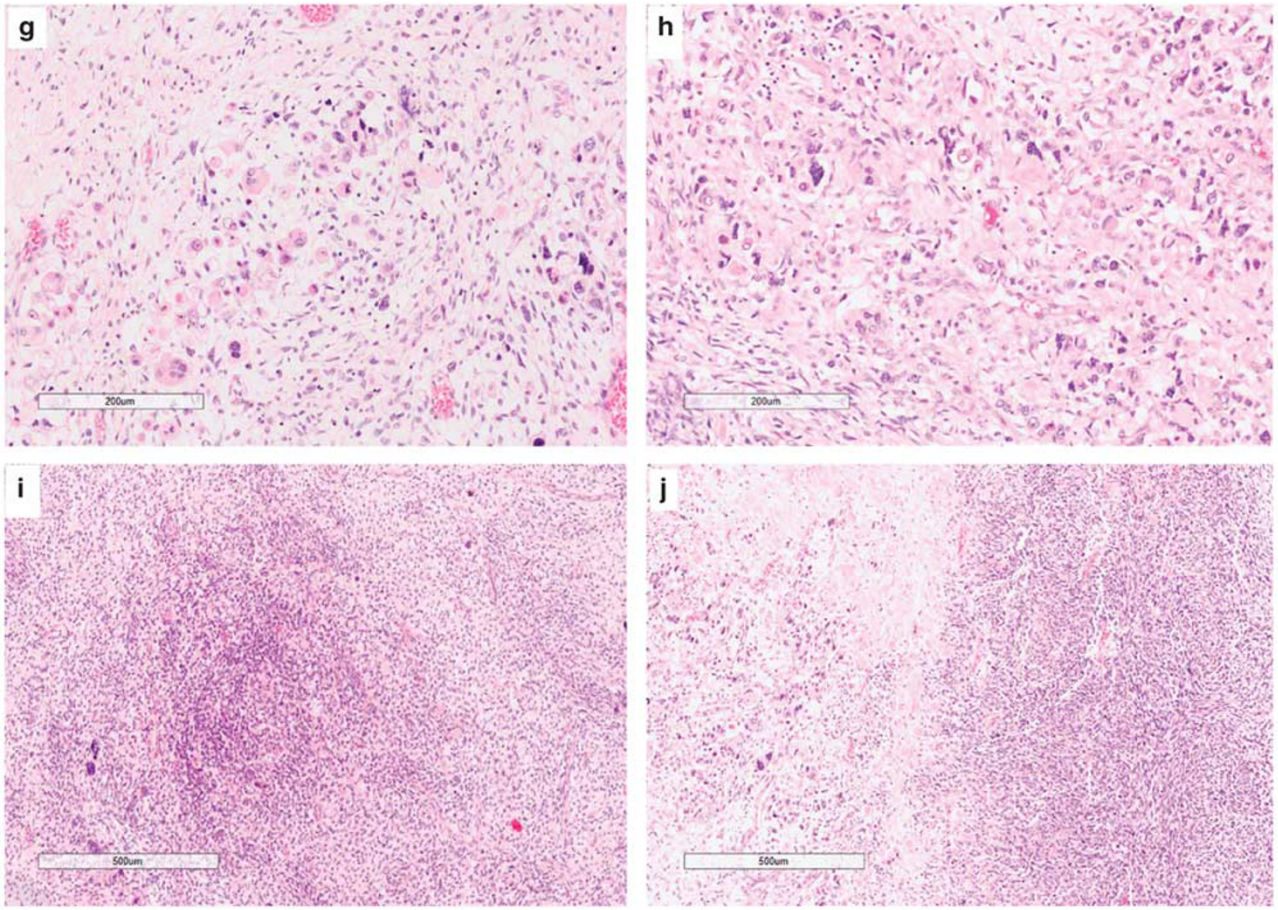


Figure 1 Continued.

8: c.5125G>A [p.D1709N]; and 8 (second hit): c.5138A>T [p.D1713V]; Figure 2). In cases 1 and 9 the identified variant c.5438A>G has the potential to cause exon skipping (p.E1788fsX41) in addition to resulting in an altered amino acid (p.E1813G).[8] Inactivating *DICER1* mutations were observed in cases 1, 6, 7, and 9 (c.5023_5025delTACinsAG [p.Y1675RfsX30]; c.2026C>T [p.R676X]; c.2062C>T [p.R688X]; and c.2450delC [p.P817LfsX15], respectively; Figure 2a). As these inactivating mutations were also observed in matched adjacent normal tissue, we deemed them to be of germline origin but cannot exclude the possibility of a mosaic origin (Figure 2a). Inactivating mutations were also detected in cases 2 (c.4684_4685inC [p.C1562SfsX34]) and 3 (c. 1630C>T [p.R544X]; Figure 2a). The inactivating mutations in these two cases are likely somatically acquired as they were not present in matched normal tissue (Figure 2a; Supplementary Table 2). Cloning experiments to determine the phase of mutations in cases 7, 8, and 9 determined that the pairs of *DICER1* mutations in each cases were most likely biallelic for cases 7 and 8, and show a trend toward biallelism in case 9 (see comment in Supplementary Data). The *DICER1* mutations in cases 1, 2, and 3 are also presumed to be biallelic based on the *DICER1* mutation phase analyses of other DICER1 syndrome lesions[13–20] but we were unable to confirm this presumption due to lack of fresh frozen tissue on which to perform cloning experiments. In summary, 8/9 anaplastic sarcomas of the kidney possessed at least one *DICER1* mutation and 7/8 contained two *DICER1* mutations (likely *in trans*, see above). In 3 of these 7 cases, both mutations seen in the tumor were of somatic origin (cases 2, 3, and 8; Figure 2b). In 4 of the 7 cases where at least one *DICER1* mutation was present, the truncating mutation was also detected in adjacent normal tissue, and therefore was likely to be either mosaic or germline in nature (cases 1, 6, 7, and 9; Figure 2b; Supplementary Table 2).

The *in vitro* cleavage data demonstrate that all the *DICER1* RNase IIIb mutations, when acting as missense mutations, are incapable of producing 5p miRNAs but instead produce 3p and an incompletely processed RNA molecule we term 5p+loop (Figure 3). If the *DICER1* RNAse IIIb mutation of cases 1 and 9 produce a protein lacking exon 25, then no miRNAs are produced.[8]

The co-occurrence of a *TP53* mutation with DICER1 was confirmed in three out of nine cases: cases 1 (c.630G>C [p.R210S]); 4 (c.212G>T [p.

R71L]); and 6 (c.521G>A [p.R174Q]). Six of nine anaplastic sarcomas of the kidney (namely, cases 1, 4, 5, 6, 8, and 9) had aberrant TP53 immunohistochemistry (Figure 4a).

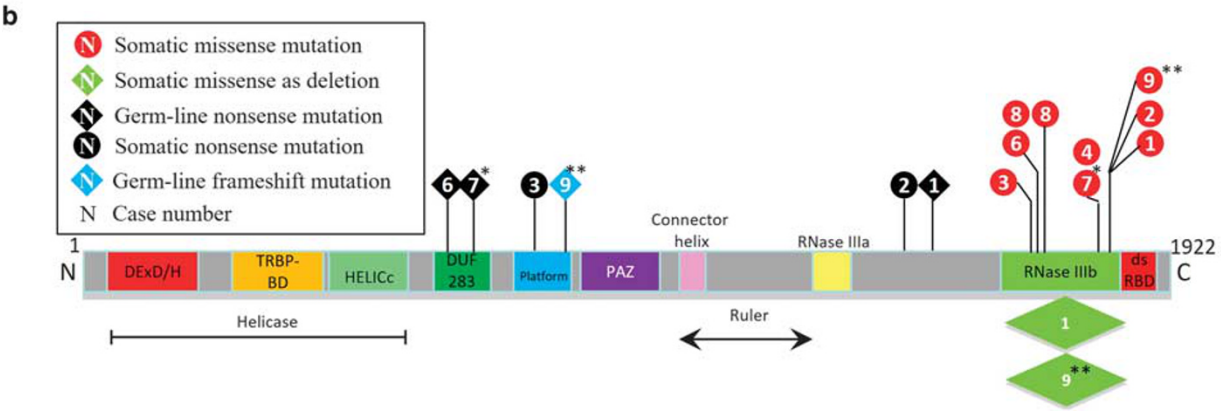
Discussion

To our knowledge, this is the largest collection of anaplastic sarcomas of the kidney from which

a

	RNAse IIIb			Inactivating mutation		
Case	mutation	tumor	normal	mutation	tumor	normal
1	c.5438A>G p.E1813G			c.5023_5025delTAC insAG p.Y1675RfsX30		
2	c.5437G>A p.E1813K			c.4684_4685insC p. C1562SfsX34		
3	c.5113G>A p.E1705K			c.1630C>T p.R544X		
4	c.5425G>A p.G1809R			undetected	undetected	undetected
5	None	None	None	n/a	None	None
6	c.5125G>A p.D1709N			c.2026C>T[e] p.R676X		failed
7*	c.5425G>A p.G1809R			c.2062C>T p.R688X		
8	c.5125G>A p.D1709N c.5138A>T p.D1713V			None	None	None
9**	c.5438A>G p.E1813G			c.2450delC p.P817LfsX15		

b



DICER1 sequencing has been attempted. There is a high prevalence of somatically acquired *DICER1* RNase IIIb mutations in our collection (8/9; Figure 2), which we suggest is an important genetic feature of the disease. Biallelic *DICER1* mutations were observed in two anaplastic sarcomas of the kidney (cases 7 and 8) and a trend toward biallelism was seen in the third (case 9) where this could be assessed. In the case of cases 2, 3, and 8, the *DICER1* mutations were both somatically acquired (Figure 2a). Therefore, the two-hit hypothesis of tumor formation is supported by the data for *DICER1* mutations.

All the somatically acquired *DICER1* RNase IIIb mutations seen in these anaplastic sarcomas of the kidney affect the cleavage of the 5p arm of the pre-miR122 stemloop (Figure 3). Using pre-miR122 as a surrogate for all DICER1-dependent pre-miRNAs, it appears that the tumors with the combination of one inactivating and one RNase IIIb *DICER1* mutations are deficient in producing 5p miRNAs and/or have deleterious effects due to the presence of 5p plus loop RNA structures as we and others have suggested in previous studies.[7,8,19,25] Alternatively, the presence of only 3p miRNAs could predispose a cell to tumorigenesis. Given the observation that inherited mutations in genes involved in miRNA processing have been observed in patients with Wilms tumor[20,26] and cystic nephroma,[27] it has been suggested that availability of a wide range of miRNAs is important for kidney development.[26] It seems clear that pediatric cystic nephroma[27] and much more rarely, Wilms tumor[20,26,28,29] can arise from the kidney of a proband with an inherited *DICER1*-inactivating mutation, but that the pathway to Wilms tumor does not appear to pass through a pre-existing pediatric cystic nephroma (Figure 5). On the other hand, our recent case report of a microscopic nascent anaplastic sarcoma of the kidney occurring in a pediatric cystic nephroma[8] (case 9 here), taken together with previous publications,[6,7] further supports the notion that an anaplastic sarcoma of the kidney can arise within a cystic nephroma (Figure 5).

Overexpressed TP53 as observed by immunohistochemistry as seen in 6/9 of the anaplastic sarcomas of the kidney (Figure 4a) has been used as a surrogate method of *TP53* mutation status. In addition, potentially damaging *TP53* mutations were identified by HaloPlex in cases 1, 4, and 6 (Figure 4b; Supplementary Table 3). We suggest that mutations in the promoter, 5′-UTR, or 3′-UTR of *TP53* not included in the Sanger sequencing and HaloPlex could account for lack of mutations detected in cases 5, 8, and 9. Nevertheless, our reported proportion of anaplastic sarcomas of the kidney with aberrant *TP53* expression by immunohistochemistry is similar with that reported for DICER1-dependent pleuropulmonary blastomas.[18]

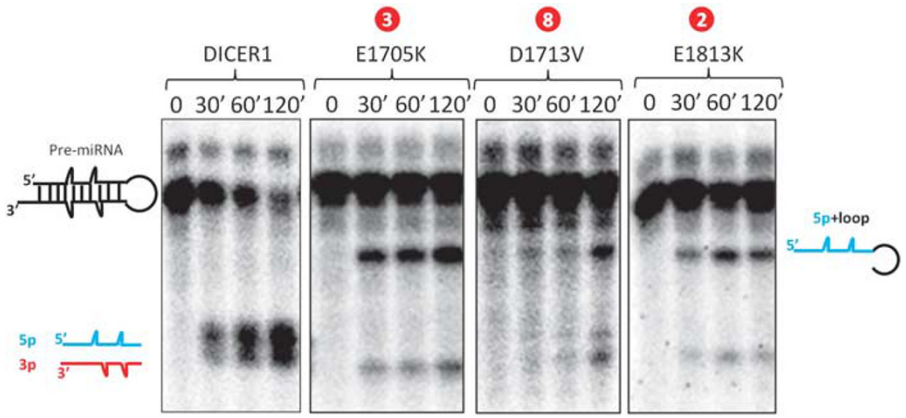


Figure 3 *In vitro* cleavage assay demonstrates that all *DICER1* RNase IIIb mutations affect the production of 5p miRNAs. Using pre-miR122 as a surrogate for all DICER1-dependent pre-miRNAs, either the presence of 5p+loop and/or absence of 5p could be responsible for altering the transcriptome to initiate aberrant cellular signaling cascades to result in anaplastic sarcoma of the kidney. The somatic mutation in case 4 (c.5425G>A) is the same as that was previously reported for case 7, which results in 5p+loop and 3p.[7] The somatic mutation (c.5425G>A) in cases 6 and 8 has been evaluated and results in 5p+loop and 3p. The somatic mutation in case 1 (c.5438A>G) is the same as that was reported for case 9, which can either result in 5p+loop and 3p (if it behaves as a missense mutation) or no miRNA generation (if it results in exon skipping).[8]

Figure 2 *DICER1* mutations in anaplastic sarcomas of the kidney. (**a**) Table showing the *DICER1* RNase IIIb mutations identified via Sanger sequencing of the hotspots or HaloPlex accompanying Sanger sequence verification. 'Undetected' = no mutation was detected by HaloPlex; 'n/a' = *DICER1* exons were not analyzed as no *DICER1* RNase IIIb mutations were identified in the case 5; 'none' = no mutation was found by Sanger sequencing (RNAse IIIb domain) or despite adequate sequencing coverage (HaloPlex for remaining DICER1 exons); 'failed' = PCR failed. (**b**) Position of mutations identified in the cases as represented as protein changes on a linear cartoon of the DICER1 protein. *This case has been previously reported.[7] **This case has been previously reported.[8] The *DICER1* mutations in cases 7 and 8 were confirmed to be biallelic by cloning (Supplementary Data). ¢ = this mutation was detected by HaloPlex in both normal and anaplastic sarcoma of the kidney tissues and we suggest that it is a germline mutation (Supplementary Table 2).

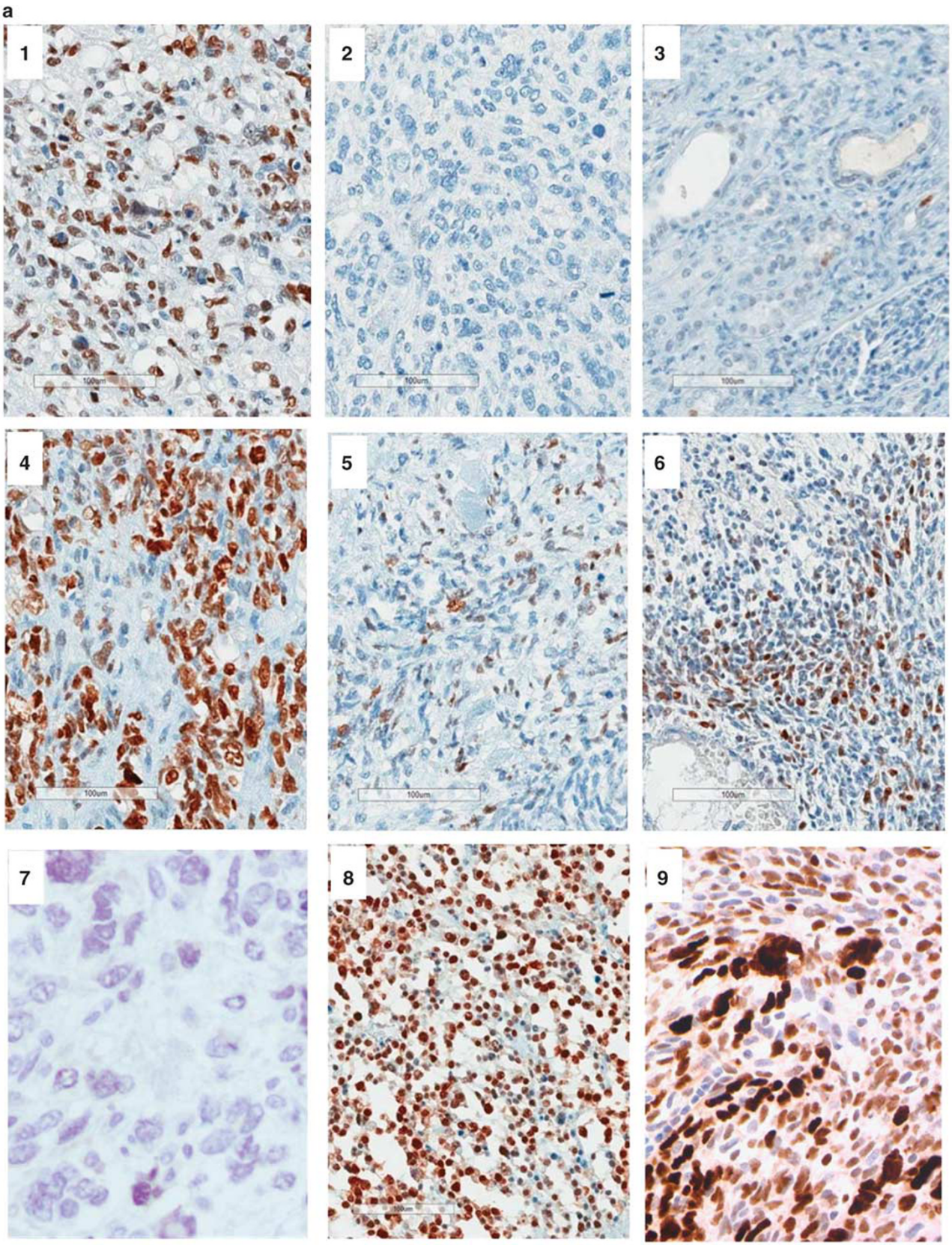


Figure 4 TP53 status is altered in a subset of anaplastic sarcomas of the kidney. (**a**) Immunohistochemistry staining. Case 1 shows strong positivity in ~50% of tumor cells; case 2 is negative; case 3 is negative; case 4 shows strong positivity in 75–80% of tumor cells; case 5 shows strong positivity in ~30% of tumor cells; case 6—strong positivity in 25–30% of tumor cells; case 7—at ×40 magnification, negative; case 8—moderate positivity in ~85% of tumor cells; case 9—over 90% of cells show very strong positivity. Scale bar is shown for cases 1–6, 8, and 9. (**b**) Damaging *TP53* mutations were identified either by HaloPlex and/or Sanger sequencing.

b

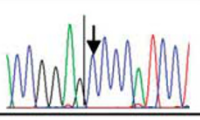
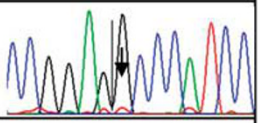
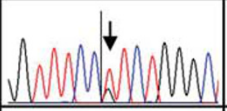
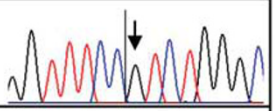
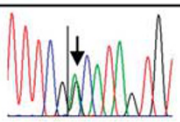
ASK	*TP53* mutation identified (method)	ASK sequence	Matched normal
1	c.630G>C (LOH) p.R210S somatic (HaloPlex)		
2	None (HaloPlex)	n/a	n/a
3	None (HaloPlex)	n/a	n/a
4	c.212G>T, rs11540654 p. R71L somatic (HaloPlex)		
5	None (HaloPlex)	n/a	n/a
6	c.521G>A p.R174Q somatic (HaloPlex)		failed
7	None (Sanger)	n/a	n/a
8	None (HaloPlex)	n/a	n/a
9	None (Sanger)	n/a	n/a

Figure 4 Continued.

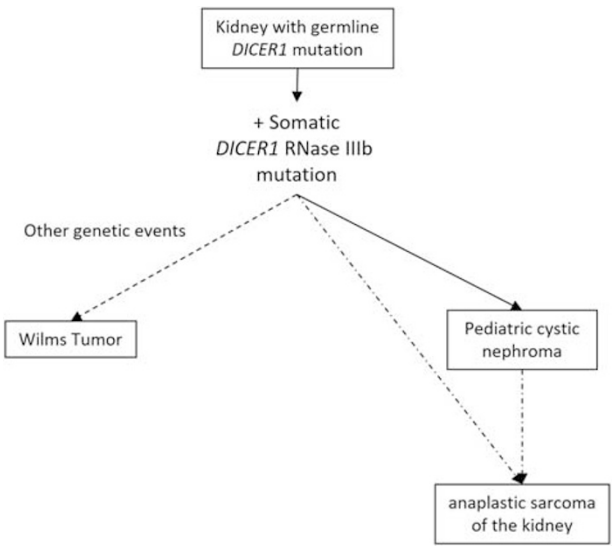


Figure 5 Model depicting possible evolution of *DICER1*-affected kidney tumors. Dotted arrows represent rare events, solid lines represent more common events, arrows with dots and dashes represent events with unknown frequencies. Wilms tumor with biallelic *DICER1* mutations is rarely observed,[20] but it seems likely that many anaplastic sarcomas of the kidney arise in pre-existing pediatric cystic nephromas.[6–8]

Case 5, which showed neither *DICER1* nor *TP53* mutations, but did overexpress TP53, shared some diagnostic features of anaplastic sarcomas of the kidney (Figures 1g–j), such as widespread anaplastic changes, but lacked some other commonly seen features such as cysts and convincing chondroid differentiation, making this case somewhat atypical and indistinguishable from anaplastic Wilms tumor. In addition, as this was the only tumor that we studied here that did not possess a *DICER1* mutation, and as *DICER1* mutations are much rarer in Wilms tumors than in anaplastic sarcomas of the kidney, we are inclined, in retrospect, to consider this to be an anaplastic Wilms tumor.

This study suggests that identification of *DICER1* RNase IIIb mutations is a useful genetic marker for anaplastic sarcomas of the kidney. Also, DICER1-dependent evolution from cystic nephroma to anaplastic sarcoma of the kidney may parallel the evolution of DICER1-dependent type I cystic pleuropulmonary blastomas to more solid types II and III pleuropulmonary blastomas. As aberrant immunohistochemistry status of *TP53* was only seen in a proportion of anaplastic sarcomas of the kidney, it is not a useful molecular marker of the disease. Given the high incidence of *DICER1* mutations in our set of anaplastic sarcomas of the kidney we suggest that screening for both germline and somatic *DICER1* mutations is warranted in suspected cases of anaplastic sarcoma of the kidney.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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