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Ovarian Tumors related to Intronic Mutations in *DICER1*: A Report from the International Ovarian and Testicular Stromal Tumor Registry

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Abstract

Germline *DICER1* mutations have been described in individuals with pleuropulmonary blastoma (PPB), ovarian Sertoli-Leydig cell tumor (SLCT), sarcomas, multinodular goiter, thyroid carcinoma, cystic nephroma and other neoplastic conditions. Early results from the International Ovarian and Testicular Stromal Tumor Registry show germline *DICER1* mutations in 48% of girls and women with SLCT. In this report, a young woman presented with ovarian undifferentiated sarcoma. Four years later, she presented with SLCT. She was successfully treated for both malignancies. Sequence results showed a germline intronic mutation in *DICER1*. This mutation results in an exact duplication of the six bases at the splice site at the intron 23 and exon 24 junction. Predicted improper splicing leads to inclusion of 10 bases of intronic sequence, frameshift and premature truncation of the protein disrupting the RNase IIIb domain. A second individual with SLCT was found to have an identical germline mutation. In each of the ovarian tumors, an additional somatic mutation in the RNase IIIb domain of DICER1 was found. In rare

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patients, germline intronic mutations in *DICER1* that are predicted to cause incorrect splicing can also contribute to the pathogenesis of SLCT.

Keywords

DICER1; Sertoli-Leydig cell tumor; miRNA; ovarian cancer; sarcoma

Introduction

Germline *DICER1* mutations were first identified in children with pleuropulmonary blastoma (PPB), a rare but aggressive lung tumor of infancy and early childhood [1, 2]. Conditions which are now known to be associated with germline *DICER1* mutations include PPB, Sertoli-Leydig cell tumor (SLCT), cystic nephroma, lung cysts, nodular hyperplasia and/or carcinoma of the thyroid gland, nasal chondromesenchymal hamartomas, embryonal rhabdomyosarcoma of the uterine cervix, ciliary body medulloepithelioma, renal sarcoma, pituitary blastoma, Wilms tumor and pineoblastoma [3–19]. DICER1 encodes an RNase III endonuclease which cleaves precursor microRNAs into active microRNAs. Most tumors in *DICER1* syndrome have one allele (often germline) harboring a nonsense or frame-shift mutation predicted to cause full loss of function (LOF) with the other allele harboring a missense mutation in the DICER1 RNase IIIb domain. This combination of mutations in both *DICER1* alleles results in improperly cleaved 5p miRNAs from the pre-miRNA hairpin structures, leading to an abnormal ratio of 5p to 3p miRNAs and altering expression of downstream target mRNAs[4]. Data from the International Ovarian and Testicular Stromal Tumor (OTST) Registry indicate that almost half of children and adults with SLCT have germline *DICER1* mutations [20]. We have also found biallelic somatic mutations in children with ovarian SLCT in absence of a germline mutation (unpublished OTST Registry data presented at Children's Oncology Group 2014 Fall Meeting). Here we report two young women with SLCT both of whom have a unique intronic loss of function germline mutation in *DICER1* and tumor-specific RNase IIIb missense mutations.

Materials and Methods

Eligible participants with a history of ovarian or testicular stromal tumors were enrolled in the International OTST Registry (www.OTSTregistry.org). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review board and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all participants or proxy caregivers. Additional written consent for this specific publication was provided by Patient #1 whose unique clinical history poses a potential for identification. This article does not contain any studies with animals performed by any of the authors. The participants described in this report were tested for germline *DICER1* mutations using previously described methodology [21]. Initial testing for *DICER1* mutations was performed by standard Sanger methods as described previously or by a commercial laboratory (Ambry Genetics, Aliso Viejo, CA). Somatic testing on formalin-fixed paraffin embedded tumor tissue as previously described [21].

Results

A 10 year old girl presented with a one month history of dysuria and pelvic pressure. She underwent exploratory laparotomy and left oophorectomy. Pathology showed an undifferentiated sarcoma with limited myogenic phenotype (Fig. 1). The tumor was characterized by moderately atypical spindle cells in a fascicular pattern with a myxoid stroma and areas of cells with anaplasia characterized by enlarged, hyperchromatic nuclei and atypical multipolar mitotic figures. The tumor lacked features of juvenile granulosa cell tumor or microscopically recognizable Sertoli-cells/tubules or Leydig cells. The tumor cells were strongly positive for vimentin and nuclear p53. Rare cells showed weak staining for desmin. Muscle specific actin and MyoD1 were negative. Inhibin was negative. She was treated with vincristine, actinomycin D and cyclophosphamide. Surveillance evaluations showed no evidence of disease until 4 years following her original diagnosis when she presented with amenorrhea and an elevated testosterone. Imaging showed a right sided ovarian mass. Pathology showed an intermediately differentiated SLCT (Fig. 2). Surgical staging showed tumor adherent to the uterus (International Federation of Gynecology and Obstetrics Stage IIA). She was treated with 6 cycles of cisplatin, etoposide and bleomycin chemotherapy. Follow-up radiographic studies showed no evidence of recurrent disease but noted 2 renal cysts. She remains alive and well 15 years following the diagnosis of undifferentiated sarcoma and 12 years following diagnosis of SLCT. Family history is negative for malignancy.

Fourteen years following her original diagnosis, she was tested for germline *DICER1* mutation. Sequence results showed an intronic mutation in *DICER1* (NM_177438.2:c.5096-12 G>A). *DICER1* next generation sequencing was performed on both of her ovarian tumors. The undifferentiated sarcoma showed the germline mutation and a somatic NM_177438.2:c.5125G>A; p.Asp1709Asn *DICER1* RNase IIIb mutation. *TP53* was also mutated in the sarcoma (NM_000546.4:c.821T>A; p.Val274Asp). Sequencing of the SLCT showed the germline mutation and a somatic RNase IIIb mutation NM_177438.2:c.5439G>T; p.Glu1813Asp. No *TP53* mutations were detected in the SLCT.

The same germline intronic mutation (c.5096-12 G>A) was detected in a second adolescent with SLCT (Fig. 3) and 2 other *DICER1*-related conditions: a lung cyst and thyroid nodule. Sequencing of this individual's SLCT showed the germline NM_177438.2:c.5096-12G>A and a somatic c.5126A>T; p.Asp1709Val. No *TP53* mutations were detected. Family History is notable for hypothyroidism in two first degree relatives and a thyroidectomy for unspecified reasons in a second degree relative. She remains alive and well more than 1 year following diagnosis of SLCT. These two individuals are not known to be related, however, detailed multi-generation pedigrees are not available.

The NM_177438.2:c.5096-12 G>A germline mutation seen in these two patients results in an exact duplication of the six bases at the splice site at the intron 23 and exon 24 junction (Fig. 4). Predicted improper splicing leads to inclusion of 10 bases of intronic sequence, frameshift and premature truncation of the protein (NP803187.1:p.Asp1699AlaFS*8) disrupting the RNase IIIb domain.

Discussion

Identification of germline *DICER1* mutations has significant clinical importance, both for the individual with the mutation indicating risk for other sites of disease, and for relatives and potential offspring who may also carry the mutation. The most urgent clinical relevance is for probands and relatives under the age of 7 years who are at risk for PPB, the most lethal tumor seen within the spectrum of *DICER1* related disorders. Early detection and treatment of cystic PPB before transformation into a solid multipatterned sarcoma may positively influence survival and reduce complications of high dose chemotherapy [22].

The finding of an underlying *DICER1* mutation also has implications for surveillance for the primary ovarian tumor. In many centers, girls and women with SLCT are followed for 2–5 years following surgery or adjuvant therapy. After that time period, they are presumed to be at low risk for recurrence. Girls and women with *DICER1* mutations remain at risk for metachronous ovarian tumors after the usual risk period for recurrence and should be followed with surveillance imaging, usually ultrasound, after the usual period for recurrence has ended. Additional screening recommendations are available at genereviews.org [6]. Genetic counseling is recommended to ensure that options for familial testing and ongoing surveillance are explored.

Intronic mutations have been described in other familial cancer predisposition syndromes including defects in mismatch repair genes and Gorlin syndrome [23–25]. Intronic mutations may in some cases create a new splice site resulting in incorporation of additional bases with or without shifting of the reading frame. In this example, a new exact replica of the splice site was created 10 bases proximal to the normal splice site. This is a unique finding in our cohort of PPB and OTST Registries. Only 7 of 90 germline mutations in children with PPB were intronic and each of these were within 2 base pairs of the splice site, the so-called canonical splice site [26]. One caveat to this study is that 12 of 124 patients with PPB did not have detectable germline *DICER1* mutations. While we predict that most of those 12 children will have tumor-specific biallelic mutations, we cannot rule out a deep intronic mutation not detected by our sequencing assay. For patients with ovarian SLCT, only preliminary analysis is available at this time. Twenty individuals have tested positive for germline *DICER1* mutations. Of these, 5 have base substitutions involving splice sites; 2 presented here and 3 others with mutations in the canonical splice site.

Conclusion

In this report, we describe the finding of a unique intronic mutation in two patients with SLCT, each with additional *DICER1*-related conditions. This mutation, which affects the splice site was not detected in SNP databases, 1000 genomes or any of the previously reported series or in other patient samples available in the International OTST or PPB Registries. In the ovarian tumors described in this report, an additional somatic mutation was found in the RNase IIIb domain.

Germline *DICER1* mutations should be suspected in individuals with a personal or family history of SLCT but also in patients with juvenile granulosa cell tumor and

gynandroblastoma or any ovarian stromal tumor or sarcoma and a personal or family history of nodular thyroid disease or thyroid carcinoma or other *DICER1* related conditions. Somatic testing of tumor tissue is the most sensitive way to detect *DICER1* mutations which may be germline or tumor-specific. When analyzing *DICER1* mutations, it is necessary to consider the role of intronic mutations creating nonfunctional DICER1 protein.

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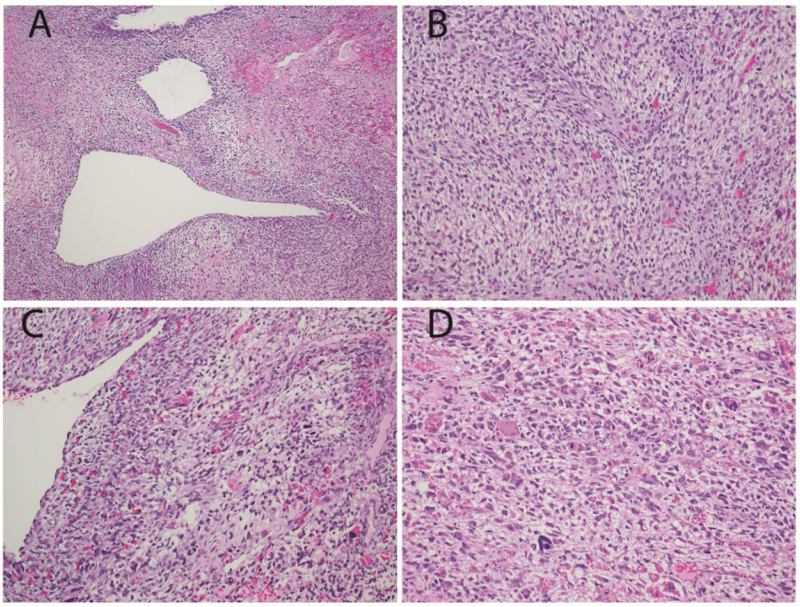

Fig. 1.

Pathologic features of the primary ovarian sarcoma in Patient #1 with *DICER1* mutation

a. Low power view shows epithelial lined cysts, solid sarcoma and focal necrosis. b. Higher power view shows spindled and stellate cells in a pale mucoid matrix. c,d. Higher power views highlight areas with significant nuclear pleomorphism. (Hematoxylin and eosin; x100 (a), x200 (b,c,d)).

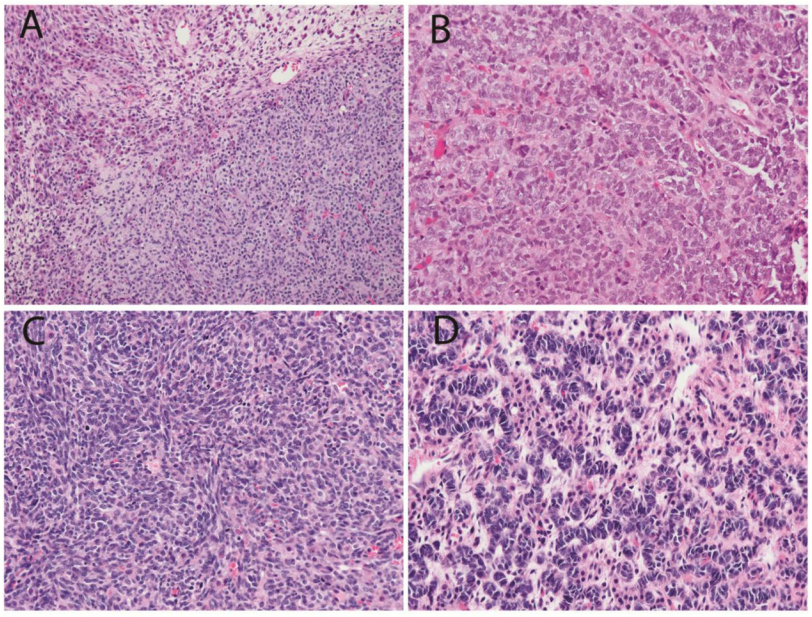

Fig. 2.

Sertoli-Leydig cell tumor in the contralateral ovary of Patient #1.

a. Low power view showing Leydig cells with abundant pink cytoplasm in upper left adjacent to nests of small Sertoli cells. b. Elongated tubules of Sertoli cells. c. Spindle cell pattern seen focally. d. Ribbons of Sertoli cells with hyperchromatic nuclei. (Hematoxylin and eosin; x100 (a), x400 (b,c,d))

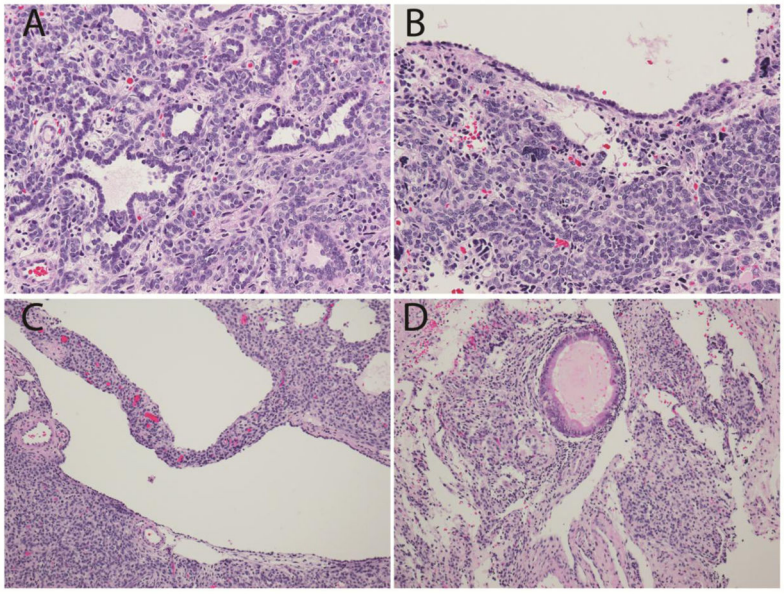

Fig. 3. Pathologic features of the Sertoli-Leydig tumor in patient #2

a.Tubular structures and nests of Sertoli cells. b. Epithelial lined cyst with nest of Sertoli cells and rare anaplastic cells. c. Cystic structure within the SLCT. d. Focal mucinous gland. (Hematoxylin and eosin; x200 (a,b), x100 (c,d)).

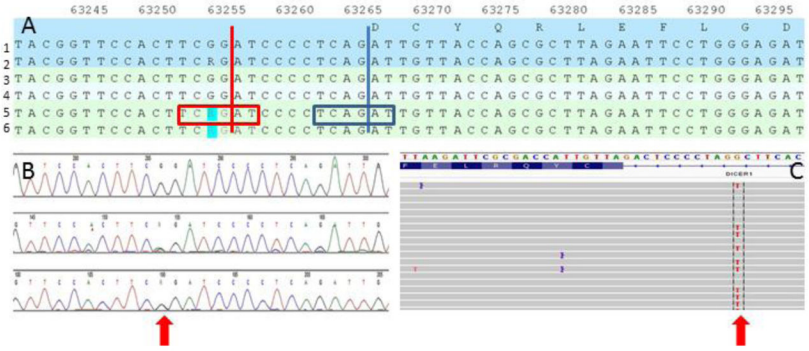

Fig. 4. Unique intronic mutation in *DICER1* resulting in duplication of splice site

a. Germline *DICER1* sequencing results from Patient #1 (Lanes 5 and 6) compared with reference genome (Lanes 1–4). Intron-exon boundary indicated by vertical blue line with six base pairs of the splice site in the blue box. Mutated base NM_177438.2:c.5096-12 G>A in blue highlight. Putative new intron-exon boundary indicated by vertical red line. b. Sanger sequence chromatogram from Patient 1. Red arrow indicates heterozygous mutation site. c. Next generation sequence data as displayed with Integrative Genome Viewer [27, 28]. Red arrow highlights the mutated base.