ORIGINAL ARTICLE

Recurrent Somatic *DICER1* Mutations in Nonepithelial Ovarian Cancers

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ABSTRACT

BACKGROUND

Germline truncating mutations in *DICER1*, an endoribonuclease in the RNase III family that is essential for processing microRNAs, have been observed in families with the pleuropulmonary blastoma–family tumor and dysplasia syndrome. Mutation carriers are at risk for nonepithelial ovarian tumors, notably sex cord–stromal tumors.

METHODS

We sequenced the whole transcriptomes or exomes of 14 nonepithelial ovarian tumors and noted closely clustered mutations in the region of *DICER1* encoding the RNase IIIb domain of DICER1 in four samples. We then sequenced this region of *DICER1* in additional ovarian tumors and in certain other tumors and queried the effect of the mutations on the enzymatic activity of DICER1 using in vitro RNA cleavage assays.

RESULTS

DICER1 mutations in the RNase IIIb domain were found in 30 of 102 nonepithelial ovarian tumors (29%), predominantly in Sertoli–Leydig cell tumors (26 of 43, or 60%), including 4 tumors with additional germline *DICER1* mutations. These mutations were restricted to codons encoding metal-binding sites within the RNase IIIb catalytic centers, which are critical for microRNA interaction and cleavage, and were somatic in all 16 samples in which germline DNA was available for testing. We also detected mutations in 1 of 14 nonseminomatous testicular germ-cell tumors, in 2 of 5 embryonal rhabdomyosarcomas, and in 1 of 266 epithelial ovarian and endometrial carcinomas. The mutant DICER1 proteins had reduced RNase IIIb activity but retained RNase IIIa activity.

CONCLUSIONS

Somatic missense mutations affecting the RNase IIIb domain of *DICER1* are common in nonepithelial ovarian tumors. These mutations do not obliterate DICER1 function but alter it in specific cell types, a novel mechanism through which perturbation of microRNA processing may be oncogenic. (Funded by the Terry Fox Research Institute and others.)

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Sex cord–stromal tumors and germ-cell tumors account for less than 10% of ovarian cancers.[1] Unlike epithelial ovarian cancers, both sex cord–stromal tumors and germ-cell tumors can also occur in the testicle; testicular germ-cell tumors are the most common cancer in boys and men of European descent between the ages of 15 and 34 years.[2,3] Other than a pathognomonic somatic mutation in *FOXL2* in adult granulosa-cell tumors,[4-6] little is known about the pathogenesis of ovarian sex cord–stromal tumors and germ-cell tumors. Recently, germline mutations in the microRNA processing gene *DICER1* have been reported in probands with pleuropulmonary blastoma or the related familial tumor dysplasia syndrome, known as pleuropulmonary blastoma–family tumor and dysplasia syndrome (Online Mendelian Inheritance in Man [OMIM] number, 601200), which includes cystic nephroma, ovarian sex cord–stromal tumor (especially Sertoli–Leydig cell tumor), and multinodular goiter.[7]

MicroRNAs (miRNAs) are a functional class of noncoding RNA molecules that regulate translation and degradation of messenger RNA.[8,9] MiRNA transcripts are processed from hairpin pre-miRNA precursors into short miRNA:miRNA* duplexes consisting of the miRNA targeting strand and the imperfectly complementary miRNA* strand (star strand, or inert carrier strand) by Dicer, an endoribonuclease with two RNase III–like domains. The RNase IIIb domain cuts the miRNA strand, whereas the RNase IIIa domain cleaves the miRNA* strand.[10,11] The resultant RNA duplex is loaded into the RNA-induced silencing complex (RISC) containing an Argonaute protein. The miRNA* strand is then removed, leaving the miRNA strand, which targets messenger RNAs for degradation or interacts with the translation initiation complex to inhibit and destabilize translation of the targeted messenger RNAs.[12,13]

The expression levels of Dicer have global effects on the biogenesis of miRNA, and reduced expression correlates with a poor outcome in many cancers.[7,14-18] In mouse models of cancer, the loss of a single *Dicer1* allele (haploinsufficiency) reduced the time to tumor onset[19] or survival time,[20] as compared with control animals. Experimental data support the hypothesis that the pathogenicity of aberrations in Dicer function is dependent on the cellular context and that the activation or inhibition of pathways for tissue-specific development and differentiation are at least partially controlled by specific miRNAs or miRNA families.[7,8,21] In mouse models with urogenital-specific knockout of *Dicer1*, there is evidence of apoptosis of germ cells and Sertoli cells[22-24] but no induction of a tumorigenic phenotype. Outside the urogenital tract, impaired differentiation occurs with Dicer loss[25-27] but requires a further challenge for oncogenesis.[19,20]

In this study, we report recurrent somatic missense mutations of *DICER1* that implicate a defect in miRNA processing, not as a permissive event in tumor onset (as might be expected for loss of function in a tumor suppressor) but rather as an oncogenic event in the specific context of nonepithelial ovarian tumors.

METHODS

EXPERIMENTAL DATA

The methodologic details of all experiments that we conducted are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

TUMOR SAMPLES

The 14 discovery samples included juvenile-type granulosa-cell tumors, primitive germ-cell tumors of the yolk-sac type, and Sertoli–Leydig cell tumors from the Children's Oncology Group at Nationwide Children's Hospital in Columbus, Ohio, and the Ovarian Cancer Research Program (OvCaRe) tissue bank in Vancouver, British Columbia, Canada. Both resources provide access to samples through specific application to studies approved by institutional review boards, including next-generation sequencing studies. We used 411 additional tumor samples for validation.

TRANSCRIPTOME, EXOME, AND SANGER SEQUENCING

We carried out transcriptome sequencing and analysis as described previously.[4,28,29] We performed exome capture of genomic coding regions through solution hybrid selection.[30] Raw data were deposited at the European Genome–Phenome Archive (accession number, EGAS00001000135). We performed Sanger sequencing in the region of *DICER1* encoding the RNase IIIb domain in DNA from samples in both the discovery and validation series and in germline DNA, when available.

DICER1 ENZYME ASSAY

We incubated wild-type DICER1 protein, mutants, and controls with 5′-^{32}phosphate (^{32}P)–labeled RNA

oligonucleotide substrates and analyzed the cleavage products by means of gel electrophoresis and phosphorimaging.

RESULTS

SOMATIC MUTATIONS IN *DICER1*

Using a combination of whole-transcriptome and whole-exome sequencing, we looked for genomic aberrations in two Sertoli–Leydig cell tumors, four juvenile granulosa-cell tumors, and eight primitive germ-cell tumors of the yolk-sac type (Tables 1 and 2 in the Supplementary Appendix). We discovered four nonsynonymous missense mutations in *DICER1* affecting the D1709 residue, one nonsense truncating mutation, and one insertion mutation that was predicted to result in a frameshift and truncation (Fig. 1). All mutations were validated by means of Sanger sequencing. The D1709 residue is within a cluster of acidic residues that are responsible for metal binding[10,11] in the catalytic center of the RNase IIIb domain (Fig. 1 in the Supplementary Appendix). Despite adequate sequence coverage, we observed no mutations that were predicted to affect the RNase IIIa domain (Fig. 2 in the Supplementary Appendix). Sequence-read data (Table 1 in the Supplementary Appendix) and Sanger traces from mutant *DICER1* samples confirmed that all mutations were heterozygous. Copy-number analysis confirmed that only a single sample, PGCTYS-05 with wild-type *DICER1*, showed heterozygous loss around *DICER1* (Fig. 3 in the Supplementary Appendix).

DICER1 MUTATIONS IN THE VALIDATION SET

The recurrent D1709 mutations in our discovery set seemed likely to have an effect on cleavage of miRNAs and metal (Mg^{2+}/Mn^{2+}) binding and are

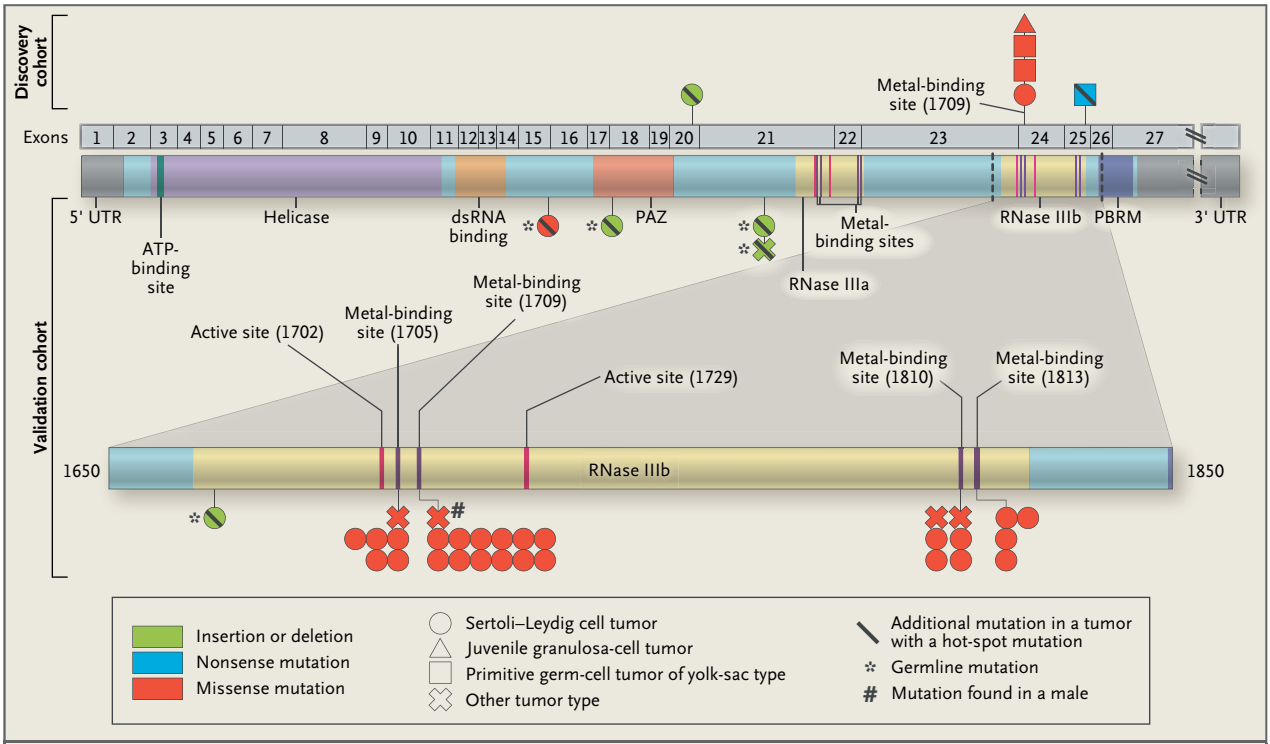

Figure 1. Mutations in *DICER1*.

The 27 exons of *DICER1* are represented as numbered gray boxes above a diagram of the gene encoding the protein (shown below in blue), with the two catalytic RNase III domains shown in yellow. Active sites are indicated by a pink line, and metal-binding sites are indicated by a purple line, with the polybromo (PBRM) domain in purple. The abbreviation dsRNA denotes double-stranded RNA, PAZ Piwi Argonaut and Zwille domain, and UTR untranslated region. The mutations that are listed above the exons were identified on whole-transcriptome or whole-exome sequencing of 14 samples in the discovery set, and those listed below were identified through targeted Sanger-sequencing analysis of 101 samples of nonepithelial ovarian tumor, 39 samples of nonepithelial testicular tumor, and 5 samples of embryonal rhabdomyosarcoma. All recurrent somatic mutations were missense mutations in codons encoding metal-binding sites in the RNase IIIb domain (magnified region). Seven of the samples were found to carry another *DICER1* mutation (indicated with a slash) in addition to a hot-spot mutation.

Table 1. Prevalence of Somatic Hot-Spot Mutations Affecting the RNase IIIb Domain of DICER1 in Nonepithelial Ovarian Tumors.*

Tumor Type	Frequency	Changes in Coding Sequence	No. of Mutations	Predicted Amino Acid Changes
	no./total no. (%)			
Sex cord–stromal tumor				
Sertoli–Leydig cell tumor	26/43 (60)	c.5113G→A	5	p.Glu1705Lys (E1705K)
		c.5125G→A†	10	p.Asp1709Asn (D1709N)
		c.5126A→G	1	p.Asp1709Gly (D1709G)
		c.5127T→A	2	p.Asp1709Glu (D1709E)
		c.5428G→C	1	p.Asp1810His (D1810H)
		c.5428G→T	2	p.Asp1810Tyr (D1810Y)
		c.5428G→A†	1	p.Asp1810Asn (D1810N)
		c.5437G→C	2	p.Glu1813Gln (E1813Q)
		c.5438A→G	1	p.Glu1813Gly (E1813G)
		c.5437G→A	1	p.Glu1813Lys (E1813K)
Juvenile granulosa-cell tumor	1/14 (7)	c.5126A→G	1	p.Asp1709Gly (D1709G)
Adult granulosa-cell tumor	0/15			
Germ-cell tumor				
Primitive germ-cell tumor				
Yolk sac	2/15 (13)	c.5125G→A	1	p.Asp1709Asn (D1709N)
		c.5127T→A	1	p.Asp1709Glu (D1709E)
Dysgerminoma	0/1			
Mixed	0/1			
Teratoma				
Immature	0/5			
Mature	1/8 (12)	c.5428G→T†	1	p.Asp1810Tyr (D1810Y)

* Listed is the frequency of hot-spot mutations in specimens in which both the 1700 and 1800 amplicons were successfully sequenced, unless otherwise indicated.

† One specimen in this group contained a somatic mutation at the indicated position but could not be assessed for mutations affecting all four RNase IIIb metal-binding sites.

thus potentially pathogenic.[10,11] We performed Sanger sequencing encompassing the regions encoding all four metal-binding residues within the RNase IIIb domain in a validation set that included 101 additional nonepithelial ovarian tumors (Table 1, and Tables 3, 4, and 5 in the Supplementary Appendix). We also screened 39 testicular tumors to determine whether they harbored the same mutations. Among the nonepithelial ovarian tumor types that were screened, mutations affecting any one metal-binding residue were present, with the highest prevalence in Sertoli–Leydig cell tumors: 26 of 43 (60%) of these tumors harbored a mutation. We designated these mutations affecting RNase IIIb metal-binding residues as hot spots. Apart from ovarian sex cord–stromal tumors and germ-cell tumors, 1 of 14 single, developmentally related, nonseminomatous testicular germ-cell tumors harbored a D1709N mutation (Table 3 in the Supplementary Appendix). To determine whether these mutations occur in other cancers with a so-called primitive phenotype, we analyzed 5 embryonal rhabdomyosarcomas; 2 of these tumors had hot-spot mutations, 1 of which occurred in a patient with a previously described germline mutation.[31]

We found hot-spot mutations in 33 of 159 tumors in the discovery and validation sets combined, including ovarian, testicular, and embryonal rhabdomyosarcoma tumor types. Among these mutations, the most prevalent were the 17 muta-

Table 2. Patients with a Hot-Spot Mutation and an Additional Germline or Somatic *DICER1* Mutation.*

Patient No.	Hot-Spot *DICER1* Mutation	Additional *DICER1* Mutation	Truncating Mutation	Mutations in Cis or Trans
ERMS-05	c.5113G→A (E1705K)	c.3907_3908delCT (germline)[31]	Yes	Not assessed
PGCTYS-02	c.5127T→A (D1709E)	c.5492G→A (somatic)	Yes	Trans
SLCT-01	c.5125G→A (D1709N)	c.3237_3238insCCAGCAT (somatic)	Yes	Trans
SLCT-08	c.5113G→A (E1705K)	c.3611_3616delACTACAinsT (germline)[31]	Yes	Not assessed
SLCT-19	c.5125G→A (D1709N)	c.5018_5021delTCAA (germline)[33]	Yes	Trans
SLCT-20†	c.5125G→A (D1709N)	c.2825delC (germline)	Yes	Trans
SLCT-26	c.5437G→A (E1813K)	c.2457C→G (germline)‡[33]	No‡	Not assessed

* ERMS denotes embryonal rhabdomyosarcoma, PGCTYS primitive germ-cell tumor of the yolk-sac type, and SLCT Sertoli–Leydig cell tumor.

† This patient presented with a family history of nonepithelial ovarian cancer on the maternal side and was therefore screened for germline mutations in *DICER1*. A germline *DICER1* mutation was identified in the proband, but the mutation was not found in her mother, and her father's DNA was not available for testing (see also Fig. 4A in the Supplementary Appendix).

‡ This mutation causes a splice change and a 21-bp in-frame deletion within the region encoding the PAZ domain. The effect of this change is unknown.

tions affecting D1709. We confirmed that mutations were somatic in 16 cases for which we had corresponding germline DNA. We attempted to assess all hot-spot regions by performing Sanger sequencing of two independent amplicons spanning the metal-binding residues (for details, see the Methods section in the Supplementary Appendix). However, because of poor preservation of DNA in formalin-fixed, paraffin-embedded material, in some instances we could sequence only a single amplicon (4 mutation-positive samples and 18 mutation-negative samples) (Table 3 in the Supplementary Appendix). In cases in which both amplicon regions were successfully sequenced, mutations affecting any one RNase IIIb metal-binding residue were mutually exclusive with mutations affecting any other IIIb metal-binding residue ($P<0.01$ by Fisher's exact tests) (Fig. 4 in the Supplementary Appendix). We observed no mutations affecting the metal-binding regions of the RNase IIIa domain, despite targeted sequencing of 4 samples that were positive for an RNase IIIb mutation and 9 samples that were mutation-negative. Immunohistochemical analysis did not provide a proxy for hot-spot mutation status, since all tumors were positive for DICER1 expression (Fig. 5 in the Supplementary Appendix).

The *DICER1* hot-spot mutations are not present in the 1000 Genomes Project data or the public-data repository of the Cancer Genome Atlas consortium. No recurrent *DICER1* mutations have been reported in the mutation database of the Catalogue of Somatic Mutations in Cancer (COSMIC), in which 4 of 938 cancers have somatic mutations but none in the RNase IIIb hot spots or RNase IIIa equivalents.[32] Moreover, these mutations were not observed in any of the more than 1000 cancer-sequencing libraries that we studied or in 15 adult granulosa-cell tumors with *FOXL2* mutations from our validation cohort. On the basis of reported DICER1 dysfunction in epithelial ovarian cancers,[14] we further analyzed a series of 266 epithelial ovarian and endometrial cancers and observed a somatic hot-spot mutation (c.5676G→A, encoding an E1813G substitution) in a single ovarian carcinosarcoma (Table 4 in the Supplementary Appendix).

COMPOUND *DICER1* MUTATIONS

Across our discovery and validation series, seven samples had a hot-spot mutation and an additional germline or somatic *DICER1* mutation (Table 2, and Table 3 in the Supplementary Appendix). To show that the specific hot-spot *DICER1* somatic mutation was a separate hit on a different allele from the germline mutation, we cloned and sequenced complementary DNA (cDNA) from sample SLCT-19, which had a germline *DICER1* deletion (5018-5021delTCAA). The primers for the

reverse-transcriptase–polymerase-chain reaction (RT-PCR) assay were chosen to allow capture of both the germline and somatic changes in a single amplicon, should the two changes exist in cis with one another (Fig. 2). Only the allele with the hot-spot mutation was observed, a finding consistent with a lymphoblastoid cell line derived from the same person in which transcripts containing the germline variant have been shown to undergo nonsense-mediated decay of messenger RNA.[33] Using this RT-PCR strategy, we further showed that hot-spot mutations in three other samples were in trans with a separate *DICER1* mutation, and expression of the allele with the hot-spot mutation was predominant (Table 2, and Fig. 6 in the Supplementary Appendix). Although it is possible that mutation of the non–hot-spot allele was present in other tumors from this study, because of the generally poor quality of DNA from formalin-fixed, paraffin-embedded samples, screening of the entire *DICER1* sequence was not performed.

FUNCTIONAL CHARACTERIZATION OF DICER1 MUTANTS

We carried out RNA cleavage assays to compare the activity of DICER1 proteins containing the hot-spot D1709N, D1709E, and E1705K substitutions with that of wild-type and control samples, including a D1709A mutant with greatly reduced RNase IIIb activity that shows total loss of DICER1 function only if a substitution in the RNase IIIa domain is also present[10,11] (Fig. 3). Wild-type DICER1 (including ectopic, endogenous, and commercially obtained forms) generated products of 18 and 22 nucleotides (Fig. 3B, and Fig. 7 in the Supplementary Appendix). These products correspond to those of enzymatic cleavage that produce a mature miRNA duplex (Fig. 3A), whereas the D1709A enzyme generated only the 18-nucleotide product, as expected.[10,11] This finding indicates that the 22-nucleotide band is derived from activity at the RNase IIIb site, whereas the 18-nucleotide band is related to activity at the RNase IIIa site. The hot-spot mutant DICER1 proteins yielded markedly low levels of the 22-nucleotide product and substantial levels of the 18-nucleotide product, suggesting that, like the D1709A mutation, the D1709N, D1709E, and E1705K substitutions result in reduced RNase IIIb activity but retention of RNase IIIa activity.

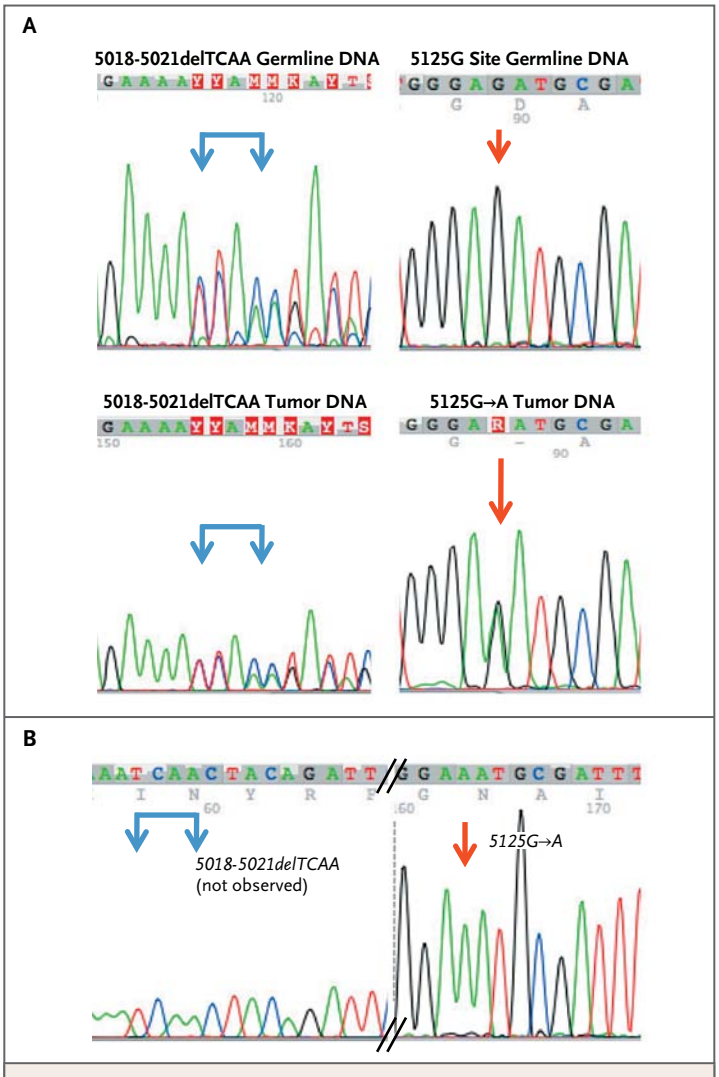

Figure 2. Germline Mutations of *DICER1* in Trans with Somatic Hot-Spot Mutations.

In Panel A, a sample from a patient with familial multinodal goiter and a Sertoli–Leydig cell tumor (SLCT-19) shows both a germline and somatic hot-spot *DICER1* mutation. The subpanels at left show sequence traces from germline (top) and tumor (bottom) samples at the site of the germline *DICER1* mutation (5018-5021delTCAA, blue arrows). The subpanels at right show the position of the hot-spot somatic *DICER1* mutation (c.5125G→A, D1709N, red arrow) from germline (top) and tumor (bottom) samples. In Panel B, a sequence trace from cloned complementary DNA in sample SLCT-19 encompasses both the germline mutation region (5018-5021delTCAA) and the somatic hot-spot mutation. Consistent with nonsense-mediated messenger RNA decay noted in lymphoblastoid cells from this patient, the germline change was also undetectable in all 98 cloned RT-PCR products from the tumor, indicating the absence of expression of this allele (see also Fig. 6 in the Supplementary Appendix). All tumors, including SLCT-19, were positive for DICER1 expression on immunohistochemical staining (see also Fig. 5 in the Supplementary Appendix).

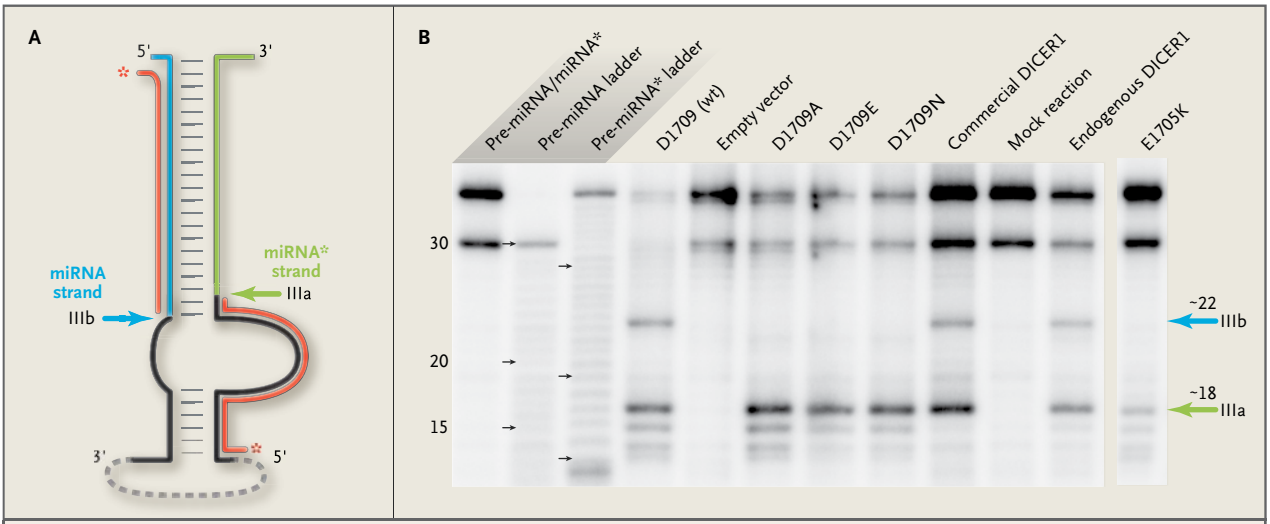

Figure 3. Functional Characterization of *DICER1* Mutations In Vitro.

Assays were performed on a pre-microRNA (miRNA) substrate that is based on the human a-3 homologue of *Caenorhabditis elegans lethal-7* (*Let-7a-3*) pre-miRNA and that permits the determination of activity in both RNase IIIb and IIIa sites. In Panel A, the pseudo pre–Let7a-3 substrate consists of two oligonucleotides with a breakpoint in the normal pre–Let7a-3 hairpin loop (dashed gray line). The blue line indicates the mature Let7a-3 miRNA released by DICER1 activity, the green line shows the miRNA star strand (miRNA*), and the red lines represent the predicted 5′-labeled (*) products. In Panel B, results of the DICER1 cleavage assay are shown. The substrates were a mixture of the 5′-^{32}P–labeled 30-nucleotide pre-miRNA strand and 39-nucleotide pre-miRNA* strand oligonucleotides. Wild-type (wt) D1709, D1709A, D1709E, D1709N, and E1705K indicate affinity-purified DICER1. Controls include empty vector, mock reaction (substrates only in Dicer enzyme assay buffer), commercial wild-type DICER1, and endogenous DICER1 (immunopurified with an anti–DICER1 antibody). The lane that is labeled pre-miRNA/miRNA* shows pre-miRNA and pre-miRNA* substrates as markers, and the lanes that are labeled pre-miRNA ladder and pre-miRNA* ladder show hydrolysis ladders of those substrates. The blue and green arrows mark the positions of the domain cleavage products of RNase IIIb (approximately 22 nucleotides) and RNase IIIa (approximately 18 nucleotides), respectively, when DICER1 binds the natural pre–Let7a-3 substrate from the left side, as in Panel A. The full gel images and additional controls are shown in Figure 7 in the Supplementary Appendix.

Preliminary analysis of miRNA expression in four Sertoli–Leydig cell tumors with *DICER1* hot-spot mutations had similar overall mature or processed miRNA expression levels as a Sertoli–Leydig cell tumor with no mutation in *DICER1* (Fig. 8 in the Supplementary Appendix). Three of these Sertoli–Leydig cell tumors had second-allele non–hot-spot mutations, two of which were known to be truncating (Table 2). Thus, miRNA processing in the latter two Sertoli–Leydig cell tumors probably occurs through the hot-spot mutant DICER1, in which case tumors with mutant and nonmutant versions of DICER1 have similar levels of miRNA-processing activity.

DISCUSSION

The hot-spot *DICER1* mutations that we report here are predominantly restricted to a specific subset of nonepithelial ovarian tumors and are highly prevalent (60%) in Sertoli–Leydig cell tumors. We also observed hot-spot mutations in a single high-grade ovarian carcinosarcoma, one testicular germ-cell tumor, and two embryonal rhabdomyosarcomas. The presence of these recurrent mutations in tumors that have an embryonal or primitive appearance suggests that their pathogenicity may be restricted to specific cell types or developmental settings.

Generally, though, somatic mutations in *DICER1* in patients with cancer are rare. A survey of cancer cell lines showed that 4 of 781 had truncating changes in *DICER1*.[34] It is not clear whether these mutations were germline or somatic, but all occurred in microsatellite-unstable lines, suggesting limited relevance to carcinogenesis. Somatic mutations in *DICER1* (albeit not the hot-spot mutations described here) have been reported in a single patient with prostate cancer[35] and in two patients with epithelial ovarian cancers.[14]

Germline truncating *DICER1* mutations have been reported in children with pleuropulmonary

blastoma or a family history of the disease and related disorders, including families with Sertoli–Leydig cell tumors[7] or ovarian sex cord–stromal tumors with or without a history of pleuropulmonary blastoma.[36] Among 823 patients with a variety of primitive tumors, there was a low prevalence of germline *DICER1* mutations that are likely to cause loss of function.[34] None of these germline mutations involved the hot-spot locations that we report here or appeared to cluster within narrow regions. Retention of the wild-type *DICER1* allele has been reported in the context of these germline loss-of-function mutations in all cases in which loss-of-heterozygosity studies were performed.[7] It is possible that undetected hot-spot mutations were present on the ostensibly wild-type allele in these tumors.

The recurrent and focal nature of the mutations and their restriction to nonepithelial ovarian tumors suggest a common oncogenic mechanism associated with a specific altered DICER1 function that is selected during tumor development in these cell types. This hypothesis is supported by several other observations. First, the in vitro biochemical data support impaired RNase IIIb activity and retention of RNase IIIa activity in tumors with hot-spot mutations. The fact that the RNase IIIa domain appears to be unaffected by mutations in the tumors that have been screened so far suggests that biologic activity in tumors with hot-spot mutations could be associated with the retained activity of the RNase IIIa site or the RISC loading function of DICER1. Second, DICER1 protein with hot-spot mutations appears to be capable of miRNA processing. We observed a second mutation in seven tumors with hot-spot mutations, and in six of these samples, the non–hot-spot mutated allele was predicted to be null. Thus, any DICER1-associated miRNA biogenesis in these six tumors was probably due to the activity of DICER1 with a hot-spot mutation. Although a global reduction in miRNA expression is one potential effect of the hot-spot mutation, our analysis suggests that a total loss of miRNA biogenesis is highly unlikely.

Finally, DICER1 expression in tumors with hot-spot mutations argues against a role for DICER1 as a classic two-hit tumor suppressor. The localized and focal pattern of mutation is typical of dominantly acting oncogenes, like *KRAS* and *BRAF*. The absence of loss of heterozygosity that is seen in association with germline *DICER1* mutations provides further evidence against a role for *DICER1* as either a haploinsufficient or a two-hit recessive tumor suppressor. Our findings suggest that *DICER1* alleles with hot-spot mutations produce viable protein. The hot-spot mutations appear to represent a new class in which the loss of the wild-type allele may occur in tandem with the retention of a hot-spot mutant DICER1 protein.

We hypothesize that an oncogenic miRNA profile is derived from altered DICER1 activity in the RNase IIIb domain. Furthermore, such activity may result in a positive bias toward the processing or selection of the RNase IIIa–processed strand of the miRNA duplex (the miRNA* strand), which is consistent with the finding that the ability of DICER1 to load miRNA into RISC does not depend on the integrity of its RNase III domains.[37] Recent studies suggest that miRNA* species may be important in gene regulation rather than simply being an inert strand.[38,39] This hypothesis is consistent with the known importance of specific miRNAs in cell differentiation and cell-fate determination, although the mechanisms by which RNase IIIb hot-spot mutations affect miRNA processing or RISC loading in the context of cancer development warrants further investigation. We conclude that in a range of nonepithelial ovarian cancers, and potentially other cancers, aberrant miRNA processing resulting from *DICER1* hot-spot mutations is a key oncogenic event.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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