Estrogen Receptor Expression in DICER1-related Lesions is Associated with the Presence of Cystic Components

Paul Scott Thorner, MD, PhD, Anne-Laure Chong, MSc,†‡ Maria Apellaniz-Ruiz, PhD,‡§‖ Naciba Benlimame, PhD,¶ Paula Marrano, MLT,# Fadi Brimo, MD,** Somruetai Shuangshoti, MD,†† Shanop Shuangshoti, MD,‡‡ and William D. Foulkes, MBBS, PhD†‡§‖*

Abstract: DICER1 tumor predisposition syndrome results from pathogenic variants in *DICER1* and is associated with a variety of benign and malignant lesions, typically involving kidney, lung, and female reproductive system. Over 70% of sarcomas in DICER1 tumor predisposition syndrome occur in females. Notably, pediatric cystic nephroma (pCN), a classic DICER1 tumor predisposition syndrome lesion, shows estrogen receptor (ER) expression in stromal cells. There are also renal, hepatic, and pancreatic lesions unassociated with DICER1 tumor predisposition syndrome that have an adult female predominance and are characterized/defined by ER-positive stromal cells. Except for pCN, the expression of ER in DICER1-associated lesions remains uninvestigated. In the present study, ER expression was assessed by immunohistochemistry in 89 cases of DICER1-related lesions and 44 lesions lacking *DICER1* pathogenic variants. Expression was seen in stromal cells in pCN and pleuropulmonary blastoma (PPB) types I and Ir, whereas anaplastic sarcoma of kidney and PPB types II and III were typically negative, as were other solid tumors of non-Müllerian origin. ER expression was unrelated to the sex or age of the patient. Expression of ER showed an inverse relationship to preferentially expressed antigen in melanoma (PRAME) expression; as lesions progressed from cystic to solid (pCN/anaplastic sarcoma of kidney, and PPB types I to III), ER expression was lost and (PRAME) expression increased. Thus, in DICER1 tumor predisposition syndrome, there is no evidence that non-Müllerian tumors are hormonally driven and antiestrogen therapy is not predicted to be beneficial. Lesions not associated with *DICER1* pathogenic variants also showed ER-positive stromal cells, including cystic pulmonary airway malformations, cystic renal dysplasia, and simple renal cysts in adult kidneys. ER expression in stromal cells is not a feature of DICER1 perturbation but rather is related to the presence of cystic components.

Key Words: DICER1, estrogen receptor, cystic nephroma, pleuropulmonary blastoma, congenital pulmonary airway malformation, cystic renal dysplasia, simple renal cyst

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DICER1 is a critical component of the microRNA (miRNA) biogenesis machinery, mainly acting in the cytoplasm to cleave hairpin precursor miRNAs to their mature forms. One of the 2 arms (5p or 3p) of the mature miRNA is loaded into the RNA-induced silencing complex and then hybridizes to targeted mRNAs, leading to posttranscriptional gene repression of silencing. DICER1 tumor predisposition syndrome (previously termed DICER1 syndrome) is a mainly pediatric-onset, autosomal dominantly inherited disorder, resulting in a characteristic array of generally rare tumors, usually with low penetrance.[1–6] Lesions most characteristic of DICER1 tumor predisposition syndrome include: pleuropulmonary blastoma (PPB),[7,8] pediatric cystic nephroma (pCN),[9,10] anaplastic sarcoma of kidney (ASK),[10,11] adolescent onset thyroid follicular nodular disease,[12] sex cord-stromal cell tumors of the ovary (especially Sertoli-Leydig cell tumors[13]), uterine cervix embryonal rhabdomyosarcoma (eRMS),[14] ciliary body medulloepithelioma,[15] nasal chondromesenchymal hamartomas,[16] pituitary blastomas,[17] pineoblastoma[18] and primary intracranial sarcoma, *DICER1*-mutant.[19] Most persons with DICER1 tumor predisposition syndrome possess a germline loss-of-function *DICER1* pathogenic variant on one allele. When tumors occur, the

From the *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; †Cancer Research Program, Research Institute of the McGill University Health Centre, Montreal, Canada; ‡Cancer Axis, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada; §Department of Human Genetics, McGill University, Montreal, Canada; ‖Gerald Bronfman Department of Oncology, McGill University, Montreal, Quebec; ¶Research Pathology Facility, Lady Davis Institute, Jewish General Hospital, Montreal, Canada; #Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto; **Department of Pathology, McGill University Health Centre, Montreal, QC, Canada; ††Institute of Pathology, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand; and ‡‡Department of Pathology and Chulalongkorn GenePRO Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

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Correspondence: William D. Foulkes, MBBS, PhD, Department of Specialized Medicine, Division of Medical Genetics, The Lady Davis Institute, Jewish General Hospital, 3755 Chemin de la Côte-Sainte-Catherine, Montreal, QC H3T 1E2, Canada (email: william.foulkes@mcgill.ca).

germline pathogenic variant is accompanied by a tumor-restricted deleterious mutation in trans, typically in exons encoding the RNase IIIb domain ("hotspot" mutations).[2,13] The resulting DICER1 protein is unable to correctly cleave the precursor miRNA, leading to impaired 5p strand production but usually maintaining 3p production.[3,8,13,20] This results in altered regulation of mRNA and appears to be a key step in oncogenesis in DICER1 tumor predisposition syndrome.

Curiously, there is a female predominance amongst patients with DICER1 tumor predisposition syndrome,[2,21,22] entirely due to the high incidence of thyroid disease and tumors of the female reproductive system. Over 70% of sarcomas occurring in DICER1 tumor predisposition syndrome occur in female patients.[5,23] In contrast, a female predominance is also known to occur in a small number of pathologic lesions, not considered to be part of the DICER1 tumor predisposition syndrome, and not involving the female reproductive system. Most are renal lesions, including adult cystic nephroma (aCN),[24–30] mixed epithelial-stromal tumor (MEST) of the kidney,[24,26,27,29–33] and angiomyolipoma with epithelial cysts;[34–36] but there are other non-renal lesions, including mucinous cystic neoplasm of the pancreas[37–45] and of the liver.[39,42,46,47] The strong predominance of female patients raises the question as to whether these lesions are, at least in part, hormonally driven. Almost all cases occur in females of reproductive age to early menopausal, in line with the idea that the lesions are hormonally stimulated. Support for this concept comes from the expression of estrogen receptors (ERs) considered to be characteristic or even diagnostic for the previously mentioned entities.[24–36,38,39,41–47] Furthermore, these studies show that it is the stromal/mesenchymal component of the lesions that expresses ER, as documented by immunohistochemistry.

Cystic nephroma is of particular relevance to DICER1 tumor predisposition syndrome. There are adult and pediatric versions of this lesion, which appear to be distinct from each other,[48,49] pCN has been associated with pathogenic variants in *DICER1*[9,10,49] but only in a single aCN case in which the pathogenic variants were at low allele frequency.[49] Although pCN shares some histologic similarities with aCN, this lesion generally occurs in young children and equally in males and females.[48] Reports of ER expression in pCN are limited. Only 2 studies have examined this, one finding all (7/7 including 6 males) cases with stroma positive for ER[49] but the other finding no cases (0/2) positive.[28] Except for these papers, there is nothing reported on ER expression in lesions associated with *DICER1* pathogenic variants.

Given the female predominance of sarcomas in DICER1 tumor predisposition syndrome, and a degree of pathologic overlap between aCN and pCN, it begs the question of whether these tumors and other lesions characteristic of DICER1 tumor predisposition syndrome express ER. Perhaps, pathogenic variants in *DICER1* can induce ER expression in stromal cells and is this sex-dependent or independent? Should ER expression be characteristic of one or more of the DICER-related malignancies or pre-malignant lesions, then might anti-estrogen therapy be a treatment option? To address these questions, this report details the expression of ER in a series of *DICER1*-mutated lesions.

MATERIALS AND METHODS

DICER1 tissue microarray (TMA), collected by the McGill University group. were as described by Thorner et al[50] and consisted of 74 lesions with confirmed *DICER1* pathogenic variants, 20 cases without, and an additional 16 normal tissue controls. Also tested (but not in the TMA) were an additional 15 cases with confirmed *DICER1* pathogenic variants, including PPB type I (n = 3), PPB type II (n = 3), PPB type III (n = 2), pCN (n = 4), and ASK (n = 3); and one case of cystic Wilms tumor with no *DICER1* pathogenic variants detected. There were also 24 additional lesions not considered to be part of the DICER1 tumor predisposition syndrome and therefore presumed to lack *DICER1* pathogenic variants but not formally tested for these. These included congenital pulmonary airway malformation (CPAM; n = 5), simple renal cyst of adult kidney (n = 16), and cystic renal dysplasia (n = 3). There were 3 cases of CPAM in the TMA, which had been tested for *DICER1* pathogenic variants and determined to be negative. The additional 5 cases of CPAM were considered to lack *DICER1* pathogenic variants, as has been shown by others.[51]

Immunohistochemistry was performed at the Segal Cancer Centre Research Pathology Facility (Jewish General Hospital; by N.B.) using the Discovery XT Autostainer (Ventana Medical System). Only staining for the α form of ER was performed, to allow comparison to the references quoted in this paper, all of which studied only ERα. Tissue samples were cut at 4μm and slides underwent de-paraffinization and heat-induced epitope retrieval (CC1 prediluted solution, reference: 950–124, standard protocol, Roche). Immunostaining for ER was performed using rabbit monoclonal anti-ER (Clone SP1, Roche) prediluted, for 32 minutes at 37°C, followed by the detection kit (OmniMap anti-Rabbit-HRP, reference: 760–4311 and ChromoMap-DAB, reference: 760–159). The negative control consisted of the omission of the primary antibody. Only nuclear staining was considered positive and scored by intensity (weak, moderate, and strong) and proportion of positive cells: 0, negative; 1+, <10% positive cells; 2+, 10% to 50% positive cells; 3+, > 50% to 90% positive cells; 4+, > 90% positive cells.[52] Any staining for ER was considered to be positive except 1+ weak. All slides were read by a single pathologist (P.S.T.).

Also, since preferentially expressed antigen in melanoma (PRAME) expression was previously found to be highly expressed in certain DICER1-associated tumors,[50] we compared the expression of this protein with ER in the same tumors to determine whether there was any relationship between the two proteins. PRAME staining results were imported from the previous report and not repeated for this study. PRAME immunostaining was performed in the same laboratory using the Discovery XT Autostainer. Slides underwent heat-induced epitope retrieval and were stained using a rabbit monoclonal

anti-PRAME, diluted at 1:200; Abcam. The PRAME antibody was applied for 32 minutes at 37°C then followed by OmniMap anti–rabbit-horseradish peroxidase and ChromoMap-diaminobenzidine. Scoring was carried out in the same manner as for ER, but tumors were only considered to be PRAME-positive if >50% of cells showed expression, in line with previously published protocols.[53,54]

RESULTS

The results of immunostaining for ER and PRAME are detailed in Table 1. Concerning ER staining of DICER1-related lesions, almost all (19/21) Sertoli-Leydig cell tumors were ER-positive, as might be predicted, given their gynecologic origin (Fig. 1A). These cases served as a positive control for the study. Another case that was ER-positive was an unusual paratesticular tumor, which was felt to be most likely of Müllerian origin.[21] Not all gynecologic tumors were positive, however. eRMS of ovary and cervix was negative for ER, but perivascular stromal cells were often ER-positive (4/6 cases). However, similar staining was also noted in vaginal eRMS (1/2), which was not DICER1-related. Many other cases showed stromal cells that were ER-positive. Of note, most cases of pCN (9/13) contained ER-positive stromal cells (Figs. 1B, C), whereas no (0/4) cases of ASK did (Fig. 1D). Similarly, most cases of PPB types I (4/7) and Ir (3/3) contained positive stromal cells (Figs. 2A, B), whereas PPB type II showed only 1/7 cases positive (and only in a cystic region; Figs. 2C, D), and no (0/5) cases of PPB type III were positive (Fig. 2E). Occasional positive stromal cells were also noted in adult pulmonary blastoma (2/3) (Fig. 2F) and nasal chondromesenchymal hamartoma (1/2). Wilms tumors with *DICER1* pathogenic variants were negative (0/2), but a cystic Wilms tumor lacking *DICER1* pathogenic variants showed positive staining of tumor cells, but only in the cystic areas of the tumor (Figs. 1E, F). Other lesions with *DICER1* pathogenic variants, including thyroid follicular nodular disease, thyroid carcinoma (follicular variant of papillary), ciliary body medulloepithelioma, intracranial spindle cell sarcoma, pineoblastoma, and cystic hepatic neoplasm, were all ER-negative.

As a comparison, additional lesions without *DICER1* pathogenic variants (proven or presumed) were also examined for ER expression. Most (13/16) simple renal cysts in adult kidneys had ER-positive stromal cells (Fig. 1G), as did all (3/3) cases of cystic renal dysplasia (Fig. 1H). Normal kidney showed occasional positive cells in the interstitium (Fig. 1I). All cases of CPAM had ER-positive stromal cells (8/8) (Figs. 2G, H). Other cases were negative including thyroid follicular nodular disease, thyroid carcinoma (a follicular variant of papillary), infantile pulmonary teratoid tumor, neuroblastoma, as well as normal lung (Fig. 2I).

The sex of the patients is relevant with respect to ER staining. Of course, positive staining for ER in gynecologic tumors showed a 100% correlation with female sex. There was one paratesticular tumor that was ER-positive but that tumor was considered to be Müllerian in origin. In contrast, examining lesions that are not considered to be sex-limited, there was no predominance of female patients in the ER-positive cases; positive cells were observed in 19 females and 23 males. Similarly, ER-negative cases were not predominantly male; there were 26 females and 31 males.

With respect to PRAME expression compared with ER expression, there was no convincing correlation between the coexpression of both proteins in DICER1-related lesions. A few lesions coexpressed ER and PRAME, including Sertoli-Leydig cell tumors (11/18 cases) and adult pulmonary blastoma (2/3), whereas lesions that were negative for both proteins included thyroid follicular nodular disease (11/11), thyroid carcinoma (a follicular variant of papillary; 2/2), intracranial spindle cell sarcoma (1/1), and neuroblastoma (1/1). For the majority of DICER1-related lesions, there seemed to be an inverse relationship between ER and PRAME expression. This was true for ciliary body medulloepithelioma, pineoblastoma, and eRMS of ovary and cervix, with expression of PRAME but not ER. Of interest are the findings for PPB and pCN/ASK. PPBs of lower stages (I and Ir) tended to express ER (7/10 cases positive) but not PRAME (1/7 cases positive), whereas in the more malignant stages (II and III), ER expression was rare (1/12 cases) but PRAME was consistently expressed (7/7 cases positive). Similarly, pCN tended to express ER (9/13 cases positive) but not PRAME (1/9 cases positive), whereas in ASK, the malignant counterpart of pCN, PRAME was expressed (1/1 case positive) but not ER (0/4 cases positive).

DISCUSSION

There are few reports on the expression of ER in DICER1-related lesions, essentially confined to two publications on pCN, with conflicting results.[28,49] The survey performed in the present study shows that ER is expressed in a variety of DICER1-related lesions but is by no means a consistent feature of lesions in the DICER1 tumor predisposition syndrome since many lesions lacked expression. One cautionary note, this study (and all studies mentioned further unless other indicated) examined the expression of ERα only. The β receptor has received much less attention and is not dealt with in the present study. Leaving aside the ovarian Sertoli-Leydig tumor, which would be expected to express ER, most of the expression of ER occurred in stromal cells of various lesions, particularly pCN and PPB. On note, ER expression decreased as lesions moved to a more malignant phenotype in both cases. Almost 70% of pCN were ER-positive, whereas ASK, the malignant counterpart of this lesion, was never positive. Similarly, 70% of PPBs of low grade (types I and Ir) were ER-positive, compared with only 8% of higher-grade tumors (types II and III). These observations imply that as lesions become more malignant, ER expression is lost. This is the opposite of PRAME expression, for which expression increased as the same lesions moved to a more malignant phenotype.[50] For pCN, only 11% of cases were PRAME-positive, whereas ASK was positive, and for

TABLE 1. Results of ER and PRAME immunostaining

Diagnosis	Number of cases	ER staining	PRAME staining	Sex
DICER1-mutated lesions				
Thyroid follicular nodular disease	11	Negative (11)	Negative (11)	4M 7F
Thyroid carcinoma (follicular variant of papillary)	2	Negative (2)	Negative (2)	2F
Ciliary body medulloepithelioma	1	Negative (1)	Positive (1)	M
Primary intracranial sarcoma, *DICER1*-mutant	1	Negative (1)	Negative (1)	M
Nasal chondro-mesenchymal hamartoma	2	Positive stroma (1)	Negative (1)	M
		Negative (1)	Negative (1)	M
Pineoblastoma	2	Negative (2)	Positive (2)	2M
Sertoli-Leydig cell tumor of ovary, moderately differentiated	13	Positive tumor (8)	Positive (8)	8F
		Positive tumor (3)	Negative (3)	3F
		Negative (1)	Positive (1)	F
		Negative (1)	Negative (1)	F
Sertoli-Leydig cell tumor of ovary, poorly differentiated	5	Positive tumor (3)	Positive (3)	3F
		Positive tumor (2)	Negative (2)	2F
Adult pulmonary blastoma	3	Positive stroma (2)	Positive (2)	2F
		Negative (1)	Positive (1)	F
PPB Type I	7	Positive stroma (1)	Negative (1)	M
		Positive stroma (3)	Not tested (3)	1M 2F
		Negative (2)	Negative (2)	1M 1F
		Negative (1)	Positive (1)	M
PPB Type Ir	3	Positive stroma (3)	Negative (3)	1M 2F
PPB Type II	7	Positive stroma in cystic area only (1)	Positive (1)	M
		Negative (3)	Positive (3)	3M
		Negative (3)	Not tested (3)	2M 1F
PPB Type III	5	Negative (3)	Positive (3)	1M 2F
		Negative (2)	Not tested (2)	1M 1F
Cystic nephroma	13	Positive stroma (1)	Positive (1)	M
		Positive stroma (7)	Negative (7)	4M 3F
		Positive stroma (1)	Not tested (1)	F
		Negative (1)	Negative (1)	M
		Negative (3)	Not tested (3)	1M 2F
Anaplastic sarcoma of kidney	4	Negative (1)	Positive (1)	F
		Negative (3)	Not tested (3)	1M 2U
Wilms tumor	2	Negative (2)	Negative (2)	2M
Cystic hepatic neoplasm	1	Negative (1)	Negative (1)	M
Paratesticular tumor of probable Müllerian origin	1	Positive tumor (1)	Negative (1)	M
Embryonal RMS of ovary	1	Negative (1)	Positive (1)	F
Embryonal RMS of cervix	5	Negative (1)	Negative (1)	F
		Positive perivascular (4)	Positive (4)	4F
DICER1-non mutated lesions				
Thyroid follicular nodular disease	8	Negative (8)	Negative (8)	4M 4F
Thyroid carcinoma (follicular variant of papillary)	2	Negative (2)	Negative (2)	2F
Sertoli-Leydig cell tumor of ovary, well differentiated	3	Positive tumor (3)	Negative (3)	3F
Infantile pulmonary teratoid tumor	1	Negative (1)	Positive (1)	F
CPAM	3	Positive stroma (3)	Negative (3)	1M 2F
Embryonal RMS of vagina	2	Positive stroma (1)	Negative (1)	F
		Negative (1)	Negative (1)	F
Neuroblastoma	1	Negative (1)	Negative (1)	F
Cystic Wilms	1	Positive tumor (cystic > > solid) (1)	Not tested (1)	M
DICER1 status unknown (control cases presumed to be negative)				
CPAM	5	Positive stroma (5)	Not tested (5)	3M 2F
Simple renal cyst	16	Positive stroma (13)	Not tested (13)	7M 3F 3U
		Negative (3)	Not tested (3)	3M
Cystic renal dysplasia	3	Positive stroma (3)	Negative (3)	1M 2F
Adult lung	2	Negative (1)	Not tested (1)	1M 1F
Adult kidney	2	Positive stroma (2)	Not tested (2)	1M 1F

CPAM, congenital pulmonary airway malformation; F, female; M, male; PPB, pleuropulmonary blastoma; RMS, rhabdomyosarcoma; U, unknown gender
Grading system is detailed in the Materials and Methods.

PPBs only 14% of types I/ Ir cases were PRAME-positive, compared with 100% of types II and III. Thus, there appears to be a reciprocal pattern of expression of ER and PRAME in these lesions. This was also true for eRMS of ovary and cervix, ciliary body medulloepithelioma, and pineoblastoma (all PRAME-positive, ER-negative). Coexpression of ER and PRAME has not been well studied in cancer, but it is known that PRAME expression in breast cancer more commonly occurs in ER-negative tumors and correlates with more malignant behavior.[55–58]

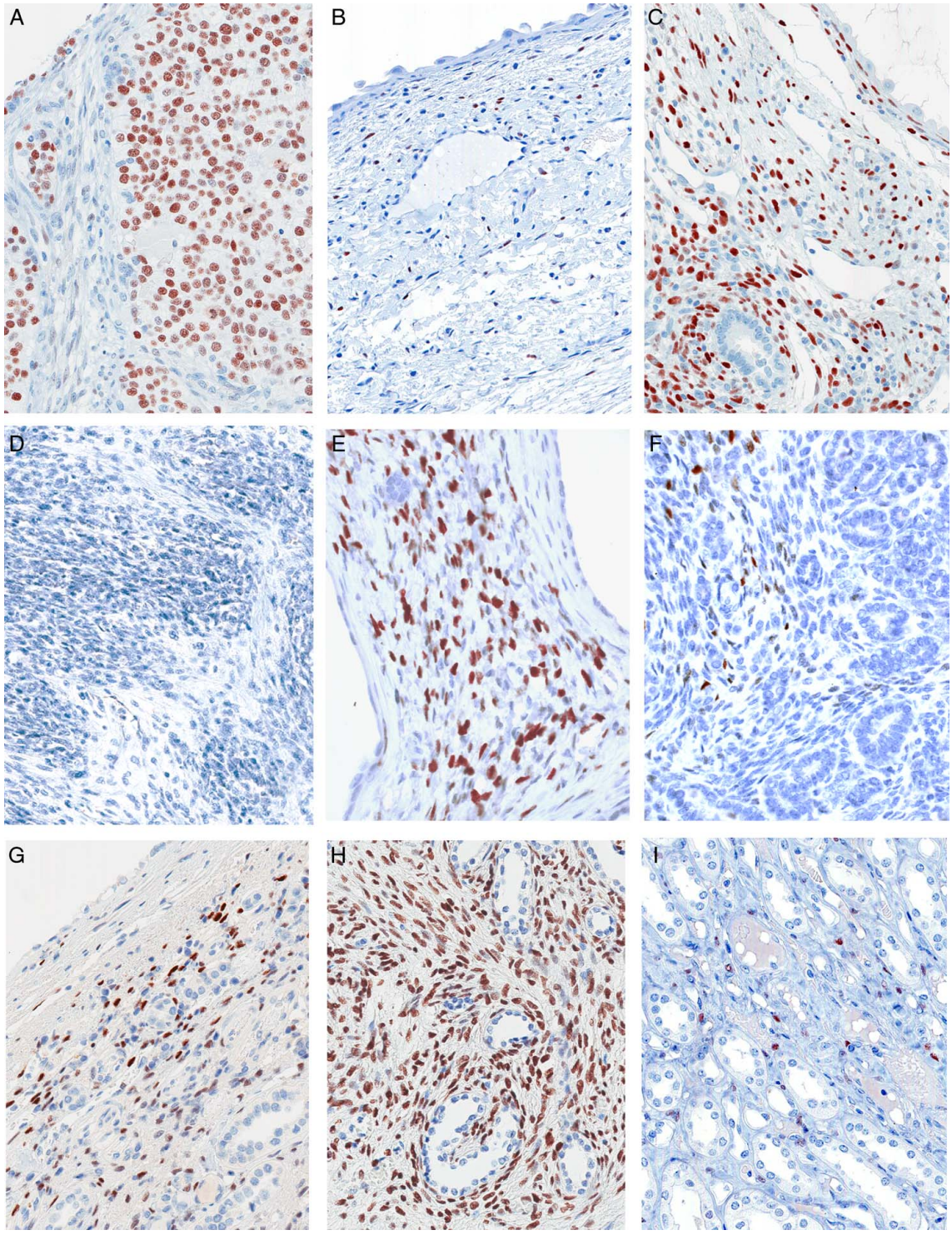

FIGURE 1. Estrogen receptor (ER) immunostaining in renal lesions. A, Sertoli-Leydig cell tumor of ovary showing diffusely positive nuclear staining of tumor cells for ER. Such cases act as a positive control. B, Pediatric cystic nephroma (pCN) showing scattered positive cells in the interstitial region between cysts. C, pCN showing numerous positive cells in the interstitial region. D, Anaplastic sarcoma of kidney showing no staining for ER. E, Wilms tumor showing numerous positive tumor cells in the stromal component between cystic regions. F, Same Wilms tumor showing only occasional positive cells in the solid region of the tumor. G, Simple renal cyst from an adult kidney showing numerous positive cells in the interstitial region adjacent to the cyst. H, Cystic renal dysplasia showing diffuse staining of the interstitial cells between the cysts. I, Normal adult kidney showing scattered positive cells in the interstitial region between tubules.

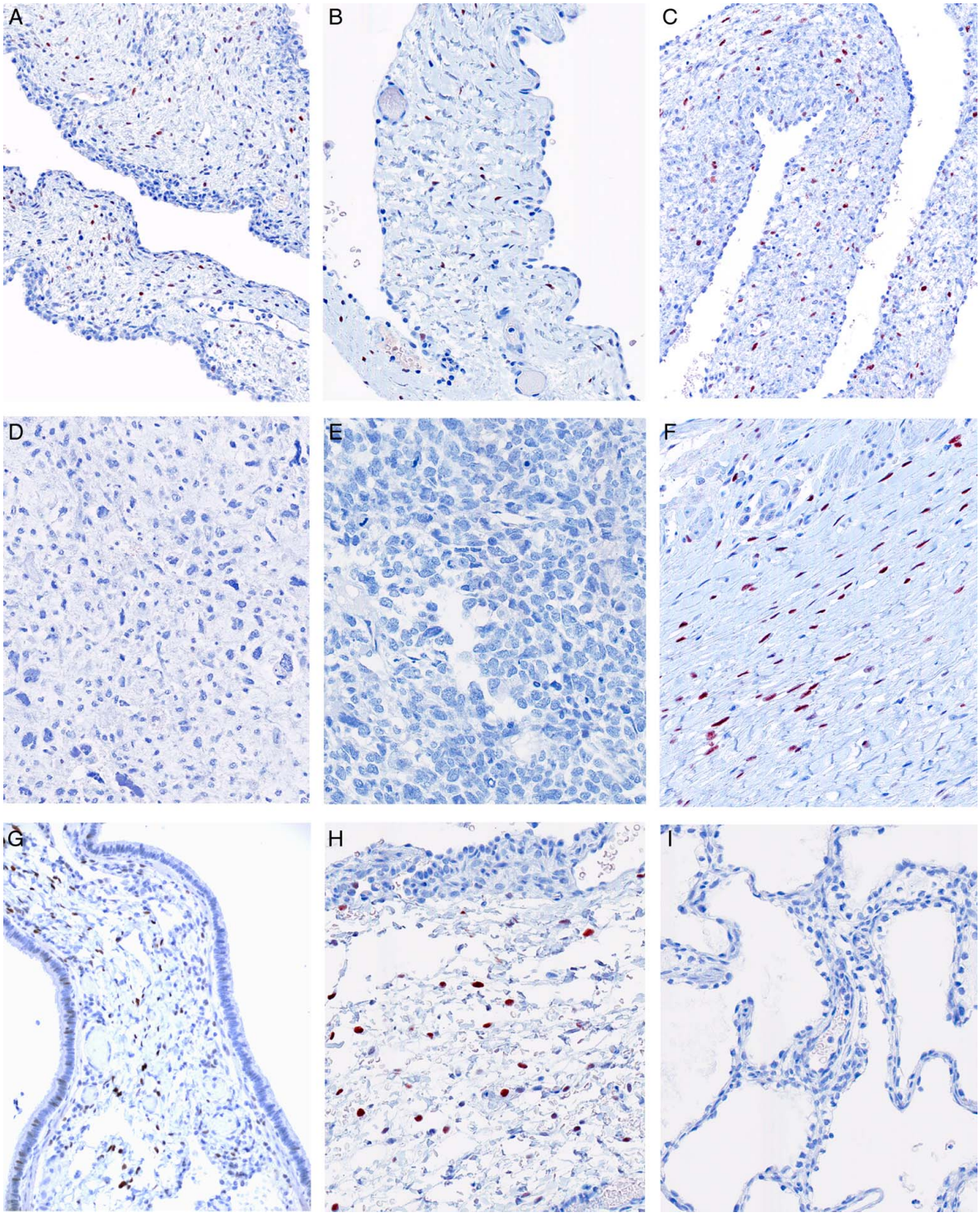

FIGURE 2. ER immunostaining in lung lesions. A, Pleuropulmonary blastoma (PPB) type I showing numerous positive cells in the interstitial region between cystic regions. B, PPB type Ir showing scattered positive cells in the interstitial region between cysts. C, PPB type II showing frequent positive cells in the interstitial region between cystic regions. D, The same PPB type II showing no positive staining in the solid region. E, PPB type III showing no staining for ER. F, Adult pulmonary blastoma showing scattered positive cells in the interstitial region. G and H, Congenital pulmonary airway malformations showing scattered positive cells in the interstitial region between cysts. I, Normal adult lung showing no staining for ER.

It is of interest to note that the ER-positive stromal cells in the DICER1-related lesions tended to occur in areas with cyst formation (Figs. 1 and 2). This was true for pCN (cystic by definition) and for PPBs, types I and Ir (purely cystic by definition). Moreover, among PPB types II and III, there was only one case of type II (mixed solid and cystic by definition) that was ER-positive, and the positive cells were present in the cystic component (Figs. 2C, D). A similar finding was noted in a case of Wilms tumor that was mainly cystic. The ER-positive cells were much more frequent in the cystic areas compared with the solid ones (Figs. 1E, F). These observations prompted a study of non-*DICER1*-mutated lesions that were cystic, including congenital lung cysts, CPAMs, simple renal cysts in adult kidneys, and cystic renal dysplasia. Almost all of the cases tested showed ER-positive stromal cells (81% of renal cysts and 100% of all the other lesions). This cannot be explained on the basis of sex; 55% of all the ER-positive cases in the study occurred in males. Similarly, 46% of all the ER-negative cases occurred in females. Hence, it would seem there is a relationship between ER-positive stromal cells and nearby cysts. As it is unlikely that the stromal cells are causing the cysts; it follows, then, that cyst formation may favor the presence of stromal cells that express ER. This concept is supported by another study on renal cysts in adults related to obstruction.[59] All cases (80% male) showed ER-positive "Müllerian" type stroma surrounding the cysts. The proposed explanation (and a reasonable one) was that the ER-positive cells reflected a metaplastic change in renal interstitial cells since no ER-positive cells were found in control cases. In the present study, occasional ER-positive cells were found in the interstitial region of the normal kidney, as has been reported by others.[60–62] Similar cells have been detected in normal lungs, although not in the present study. These cells may be involved in organ development during fetal life, but their role in post-natal life is not well understood. With respect to a role in cystic lesions, mechanical pressure on mesenchymal cells promotes ER expression, cell proliferation, and F-actin stress fiber formation.[63] Similarly, experimental ureteral ligation leads to fibroblast activation and extracellular matrix deposition in the kidney through the TGFβ1 signaling pathway, and activation of that pathway occurs through ER alpha receptors.[64] A plausible sequence, then, for the cystic lesions in the present study is that mechanical stretch from nearby enlarging cysts promotes the presence of the pericystic ER-positive stromal cells, and the stromal cells in turn provide tissue support for the cysts.

Cystic lesions with ER-positive stroma that are not gynecologic and not DICER1-related are known to occur in adults, involving the kidney, pancreas, liver, and lung. Renal lesions include aCN,[24–30] and the related lesion, MEST of the kidney,[24,26,27,29–33] as well as angiomyolipoma with epithelial cysts.[34–36] There is also mucinous cystic neoplasm of the pancreas[37–45,65] and of the liver.[39,42,46,47] A common theme in these lesions is a female predominance and reference to the stroma around the cysts as being "ovarian" or "Müllerian" in appearance. The positive expression of ER in these stromal cysts is interpreted as supportive of this concept. It is speculated that the stroma is uncommitted mesenchyme in the kidney, pancreas, and liver that becomes "ovarian" in nature under hormonal stimulation.[24,29,31,34,44,66] A less likely explanation is that these cells reflect abnormal migration of ovarian stromal cells during embryogenesis, which proliferate later in life under hormonal stimulation[29,45] as this would not account for why such lesions sometimes occur in males (and are also ER-positive), nor why these lesions do not occur in the ovary. Furthermore, in the present study, the ER-positive DICER1-associated lesions occurred slightly more often in males, and none of the patients were adults. Thus, hormonal stimulation is unlikely to be involved in the pathogenesis of these lesions, and any resemblance of the stroma to "ovarian" or "Müllerian" would just be on a descriptive basis. We cannot refute, however, that the previously mentioned adult lesions (aCN, MEST of the kidney, angiomyolipoma with epithelial cysts, mucinous cystic neoplasm of the pancreas and liver) may have a hormonal component to their pathogenesis.

The role of ER-positive cells in malignant progression in DICER1-related lesions is not clear. In general, progression from an ER-positive phenotype to an ER-negative phenotype involves the activation of growth-promoting signals, correlating with increased mitogen-activated protein kinase activity,[67] but to what extent this is relevant to DICER1-related malignancies is not known. Malignant progression in DICER1 tumors is usually associated with a transition from cystic to solid and, presumably, the cystic components are overgrown by the sarcomatous component. This change is associated with a loss of ER expression and an increase in PRAME expression. It is unclear if the same or different cells express ER and PRAME in DICER1-related lesions as the positive cells are scattered amongst negative cells. If the cases of cystic renal dysplasia in this study can be used to shed light on this question, these cases showed diffuse strong expression of ER in stromal cells, yet were completely PRAME-negative, indicating co-expression is not occurring, at least in this setting.

A female predominance in a pathologic lesion often prompts a search for expression of ER, but the converse investigation is seldom performed, namely ER expression in lesions that do not show female predominance. Our study on DICER1-related lesions shows that a large proportion of lesions that are cystic, in particular, pCN and PPB, do express ER, yet there is no female predominance and most patients are children. Thus, there is no evidence the lesions are hormonally driven and it is, therefore, unlikely that such lesions would benefit from anti-estrogen therapy. In fact, as these lesions progress, ER expression disappears while PRAME expression increases, making the latter a more attractive therapeutic target. It is also worth noting that cystic lesions of lung and kidney that are not DICER1-related, also commonly express ER. Thus, ER expression seems intimately connected to cyst formation, and unrelated to *DICER1* pathogenic variants or patient sex or age. Curiously, the ER-expressing cells are

stromal, rather than epithelial. We postulate that these stromal cells are a reaction to cyst formation that is likely to result in mechanical stretching of surrounding tissue. Such cells could reflect an expansion of ER-positive cells naturally occurring in the organ or a metaplastic response of proliferating stromal cells. The reason for ER expression in these cells is not clear and requires further study.

CONCLUSION

ER expression is present in some DICER1-related lesions, but studies of similar but DICER1-unrelated lesions prove that this expression is not a feature of DICER1 perturbation but rather is related to the presence of cystic components.

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