Recurrent *DICER1* Hotspot Mutations in Malignant Thyroid Gland Teratomas

Molecular Characterization and Proposal for a Separate Classification

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Abstract: Thyroid gland teratomas are rare tumors that span a wide clinicopathologic spectrum. Although benign and immature teratomas arise in infants and young children and generally have good outcomes, malignant teratomas affect adults and follow an aggressive course. This divergent behavior raises the possibility that benign/immature and malignant teratomas are separate entities rather than different grades of a single tumor. However, the histogenesis and molecular underpinnings of thyroid gland teratomas are poorly understood regardless of grade. In this study, we performed next-generation sequencing on 8 thyroid gland teratomas, including 4 malignant, 3 benign, and 1 immature. We identified *DICER1* hotspot mutations in all 4 malignant cases (100%) but not in any benign/immature cases (0%). No clinically significant mutations in other genes were found in either group. We also performed immunohistochemistry to characterize the primitive components of malignant teratomas. Not only did all cases consistently contain immature neural elements (synaptophysin and INSM1 positive), but also spindled cells with rhabdomyoblastic differentiation (desmin and myogenin positive) and bland epithelial proliferations of thyroid follicular origin (TTF-1 and PAX8 positive). Although *DICER1* mutations have previously been implicated in multinodular hyperplasia and well-differentiated thyroid carcinomas, these findings demonstrate the first recurrent role for *DICER1* in primitive thyroid tumors. The combined neural, rhabdomyoblastic, and homologous epithelial elements highlighted in this series of malignant thyroid gland teratomas parallel the components of *DICER1*-mutated tumors in other organs. Overall, these molecular findings further expand the differences between benign/immature teratomas and malignant teratomas, supporting the classification of these tumors as separate entities.

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BACKGROUND

In the thyroid gland, teratomas are extremely rare tumors that span a wide clinical and pathologic spectrum. As in other anatomic sites, diagnosis of thyroid gland teratoma is broadly defined by the presence of tissues derived from all 3 embryonal layers, that is, ectoderm, mesoderm, and endoderm. Within this category, these tumors are graded as benign, immature, and malignant based on the histologic fraction of immature neuroectodermal components they contain,[1] a distinction that separates them into 2 divergent demographic and prognostic groups. Benign and immature thyroid teratomas almost exclusively arise in infants and young children, including a significant subset of tumors that occurs congenitally.[2] While they can cause morbidity and even mortality due to compression of vital structures, this subset of tumors has an excellent prognosis when completely excised.[2–4] In contrast, malignant thyroid gland teratomas generally occur in older children and adults. These are aggressive tumors that can give rise to locally infiltrative growth, widespread metastasis, and death from disease, although good outcomes can be achieved through intensive multimodality therapy.[2,5]

Given the vast differences in clinical presentation and outcomes between benign/immature and malignant thyroid gland teratomas, it is not clear whether these categories represent different points on the same spectrum or entirely distinct entities. However, the histogenesis and molecular underpinnings of teratomas that arise in the thyroid gland are poorly understood regardless of tumor grade or patient characteristics. Thyroid teratomas are formally classified as germ cell tumors and are thought to develop from misplaced embryonic rests.[1] But unlike teratomas of gonadal or sacrococcygeal sites, they have never been reported to occur in conjunction with other germ cell tumor types.[6] Furthermore, rare studies that have performed cytogenetic evaluation of thyroid gland teratomas have failed to identify the isochromosome 12p characteristic of the vast majority of testicular teratomas and a subset of teratomas

at nontesticular sites.[7–12] This study aims to help better understand the pathogenesis and classification of thyroid gland teratomas via next-generation sequencing (NGS).

METHODS

Case Selection

We identified 8 cases of thyroid gland teratoma from the surgical pathology archives of The Johns Hopkins Hospital and the authors' consultation files between 2009 and 2018. Similar to previous literature,[2] tumors were selected for inclusion in this study if they contained tissues from all 3 embryonal layers and arose in the anterior neck, regardless of whether thyroid tissue was evident in the specimen. Patient demographics and clinical history were obtained from the electronic medical record. All available slides were reviewed for each case, and specific recognizable histologic components were documented. The grade was principally assigned based on the presence of immature neural tissues, with mature teratomas containing only mature neural elements, immature teratomas including immature neural elements occupying <4 low-power fields, and malignant teratomas having immature neural elements in ≥4 low-power fields.[2] These metrics were evaluated using a ×4 UPlanFLN objective with a ×10 WHN ocular on an Olympus BX41 microscope (Olympus America, Melville, NY). Teratomas were also classified as malignant if they contained a predominance of primitive non-neural elements that demonstrated hypercellularity, increased mitoses, and cytological atypia, regardless of immature neural content.

Immunohistochemistry

Immunohistochemistry was performed on all malignant teratomas to better characterize their primitive elements. Whole-slide sections were cut at 4 μm from formalin-fixed paraffin-embedded tissue blocks. All staining was performed using standardized automated protocols on a Ventana BenchMark Ultra autostainer (Ventana Medical Systems, Tucson, AZ) in the presence of appropriate controls. Mouse monoclonal antibodies were applied for AE1/AE3 (clone PCK-26; Ventana; prediluted), TTF-1 (clone 8G7G3; Ventana; prediluted), PAX8 (polyclonal; BD Pharmingen, San Diego, CA; 1:800 dilution), S100 (clone 4C4.9; Ventana; prediluted), synaptophysin (clone 27G12; Leica Biosystems, Buffalo Grove, IL; 1:400 dilution), INSM1 (clone A8; Santa Cruz Biotechnology, Dallas, TX; 1:200 dilution), desmin (clone D33; Dako, Carpinteria, CA; 1:100 dilution), myogenin (clone F5D; Ventana; prediluted), and CD99 (clone 12E7; Abcam, Cambridge, MA; 1:200 dilution). Signals were visualized using the ultraView polymer detection kit (Ventana).

Next-generation Sequencing

Formalin-fixed paraffin-embedded tumor tissue was used for molecular analysis in all cases. NGS was performed on 6 cases at the University of Texas Southwestern Medical Center as previously described.[13] Briefly, DNA was isolated using Qiagen AllPrep kits (Qiagen, Germantown, MD) and custom NimbleGen probes (Roche, Indianapolis, IN) were used to create an enriched library containing all exons from >1425 cancer-related genes. Sequencing was performed on the NextSeq. 550 (Illumina, San Diego, CA) with a median target exon coverage of ×900. An additional 2 cases underwent NGS at The Johns Hopkins Hospital as described in detail elsewhere.[14,15] In short, DNA was isolated using the automated Siemens Tissue Preparation System (Siemens Healthcare Diagnostics, Tarrytown, NY), and libraries containing the full coding regions of 644 cancer-associated genes were prepared using the SureSelect XT Target Enrichment System (Agilent Technologies, Santa Clara, CA). Sequencing was performed to an average of ×500 to ×1000 read depth on the HiSeq 2500 platform (Illumina). For all cases, variants were reviewed using the Integrated Genomics Viewer (Broad Institute, Cambridge, MA) and annotated using the gnomAD, dbSNP and/or COSMIC databases.

RESULTS

Clinical and demographic details are summarized in Table 1. A total of 3 benign, 1 immature, and 4 malignant thyroid gland teratomas were identified for inclusion in this study. The benign and immature teratomas were taken from 3 females and 1 male with a mean age of 5 months (range: 2 wk to 18 mo), while the malignant teratomas represented 3 females and 1 male with a mean age of 49 years (range: 29 to 65 y). One patient with a

TABLE 1. Clinical and Demographic Information

Case No.	Age	Sex	Size (cm)	Presentation	Grade	Treatment	Metastasis	Follow-up (mo)	Status
1	65 y	Female	1.9	Neck mass	Malignant	Surgery, chemo	Level VI LN	125	NED
2	29 y	Female	10	Neck mass	Malignant	Surgery, chemo, XRT	Level VI LN, para-aortic, clavicle	53	DOD
3	42 y	Female	8	Neck mass	Malignant	Surgery, chemo, XRT	None	64	NED
4	60 y	Male	1.7	Incidentally found on imaging	Malignant	Surgery	Level VI LN, widespread skeletal	10	DOD
5	1 mo	Male	5.9	Congenital neck mass	Immature	Surgery	None	73	NED
6	7 mo	Female	7.3	Congenital neck mass	Benign	Surgery	None	54	NED
7	18 mo	Female	4.5	Congenital neck mass	Benign	Surgery	None	19	NED
8	2 wk	Female	2.3	Congenital neck mass	Benign	Surgery	None	63	NED

Chemo indicates chemotherapy; DOD, dead of disease; LN, lymph node; NED, no evidence of disease; XRT, external-beam radiation therapy.

malignant teratoma also had multiple cutaneous basal cell carcinomas, but no other patients carried a significant cancer history. All 4 patients with benign or immature teratomas presented with congenital neck masses, with a mean size of 5 cm (range: 2.3 to 7.3 cm). Three of the patients with malignant teratomas presented with painless palpable neck masses, while 1 malignant teratoma was found incidentally on imaging during workup for hyperparathyroidism; these tumors had a mean size of 5.4 cm (range: 1.7 to 10 cm). Four malignant, 1 immature, and 1 benign teratoma arose within the thyroid gland, while 2 benign teratomas involved central neck soft tissue without identifiable normal thyroid gland tissue.

Histologically, the benign and immature teratomas contained a broad admixture of tissue elements that were all easily recognizable on hematoxylin and eosin sections. All benign and immature tumors demonstrated mature glial tissue, cartilage, bone, skeletal muscle, smooth muscle, and respiratory mucosa in varying proportions (Fig. 1A). A subset of benign teratomas also included mature skin, stomach, small bowel, colon, ciliary body, retina, choroid plexus, liver, and pancreas tissue. In 2 cases, respiratory and colonic epithelial elements, respectively, focally paired with cartilage and smooth muscle in an organoid configuration that recapitulated normal tissue architecture (Fig. 1B). The immature teratoma displayed additional immature neural elements that occupied <1 low-power field but no other primitive components (Fig. 1C). This immature neural tissue was cytologically bland without necrosis or an elevated mitotic rate (Fig. 1D).

In contrast, all malignant teratomas were predominantly composed of primitive and undifferentiated elements including immature neural tissue, spindled cells, and epithelial proliferations (Fig. 1E). These elements were present in varying proportions, with 2 malignant teratomas showing a predominance of immature neural tissue that occupied >4 low-power fields and 2 showing only focal neural tissue with a predominance of hypercellular primitive spindled and epithelial elements (Fig. 1F). The neural tissue displayed sheets and rosettes of undifferentiated cells with hyperchromatic nuclei and scant neuropil (Fig. 2A). Regardless of extent or morphology, these areas demonstrated weak synaptophysin (Fig. 2B) and patchy INSM1 positivity (Fig. 2C) and were negative for CD99. The spindled elements were composed of sheets of primitive mesenchymal cells with hyperchromatic nuclei and variable amounts of eosinophilic cytoplasm (Fig. 2D); these cells uniformly displayed positivity for desmin (Fig. 2E) and myogenin (Fig. 2F), consistent with rhabdomyoblastic differentiation. The epithelial structures included tubular and cribriform proliferations of bland cuboidal to columnar cells with clear to eosinophilic cytoplasm and no colloid production (Fig. 2G); they were strongly positive for AE1/AE3, TTF-1 (Fig. 2H), and PAX8 (Fig. 2I), suggestive of thyroid follicular origin. Despite their thyroid follicular differentiation, these epithelial proliferations had complex architecture, immature cytology, and consistent close association with other primitive tissue types that were more in keeping with neoplastic elements rather than entrapped normal or hyperplastic follicles. Immature cartilage was also a prominent component of 3 malignant teratomas, while mature skin, skeletal muscle, and smooth muscle were each focally identified in 1 case.

Targeted NGS results are summarized in Table 2. NGS was successfully performed on 4 malignant, 2 benign and 1 immature teratoma; 1 benign teratoma could not be sequenced due to inadequate DNA quality. All 4 malignant teratomas (100%) harbored hotspot *DICER1* mutations previously reported to be pathogenic.[16–29] In 3 of 4 malignant teratomas, nonhotspot *DICER1* mutations were also identified. Although these mutations have not been previously reported, they include 1 nonsense and 2 frameshift mutations that likely serve as loss of function mutations, suggestive of biallelic disruption of normal protein function. No other known oncogenic driver mutations were identified in malignant teratomas, although 2 tumors showed copy number abnormalities including chromosome 1p loss and 1q gain and chromosome 2 amplification, and several variants of uncertain significance were documented in each tumor. The 2 benign and 1 immature teratoma sequenced did not show evidence of *DICER1* hotspot mutations (0%), although 1 case of benign teratoma did have a nonhotspot *DICER1* missense mutation that also was previously unreported and is of unknown pathogenic significance. The benign and immature teratomas likewise lacked other known oncogenic driver mutations, although 2 cases also contained variants of uncertain significance.

Treatment and follow-up information is also noted in Table 1. All benign, immature, and malignant teratomas were initially treated with surgery. Central neck lymph node metastases were present at the time of resection in 3 of the malignant teratomas (75%). Adjuvant chemotherapy was administered in 3 malignant teratomas (75%) and external-beam radiation therapy in 2 malignant teratomas (50%); 1 patient did not receive adjuvant therapy due to numerous medical comorbidities. Follow-up intervals ranged from 10 to 125 months with a median of 58.5 months. Two patients with malignant teratomas developed distant metastases, 1 to para-aortic soft tissue at 17 months and clavicular bone at 51 months and 1 with widespread skeletal disease at 6 months. Both of these patients died of disease at 53 and 10 months, respectively, while all other patients had no evidence of disease at last available follow-up.

DISCUSSION

Teratomas that arise in the thyroid gland are an extremely diverse group of tumors, with benign/immature teratomas arising exclusively in infants and young children and having good outcomes and malignant teratomas largely occurring in adults and following an aggressive course.[2] The vast difference in clinical presentations and oncologic outcomes between benign/immature and malignant teratomas raise the possibility that these different grades of tumor actually represent distinct entities. Although thyroid teratomas are broadly classified as germ cell tumors, their histogenesis and molecular underpinnings are poorly understood regardless of grade. In this study, we performed NGS on a group of benign, immature, and malignant teratomas to better understand their development and classification.

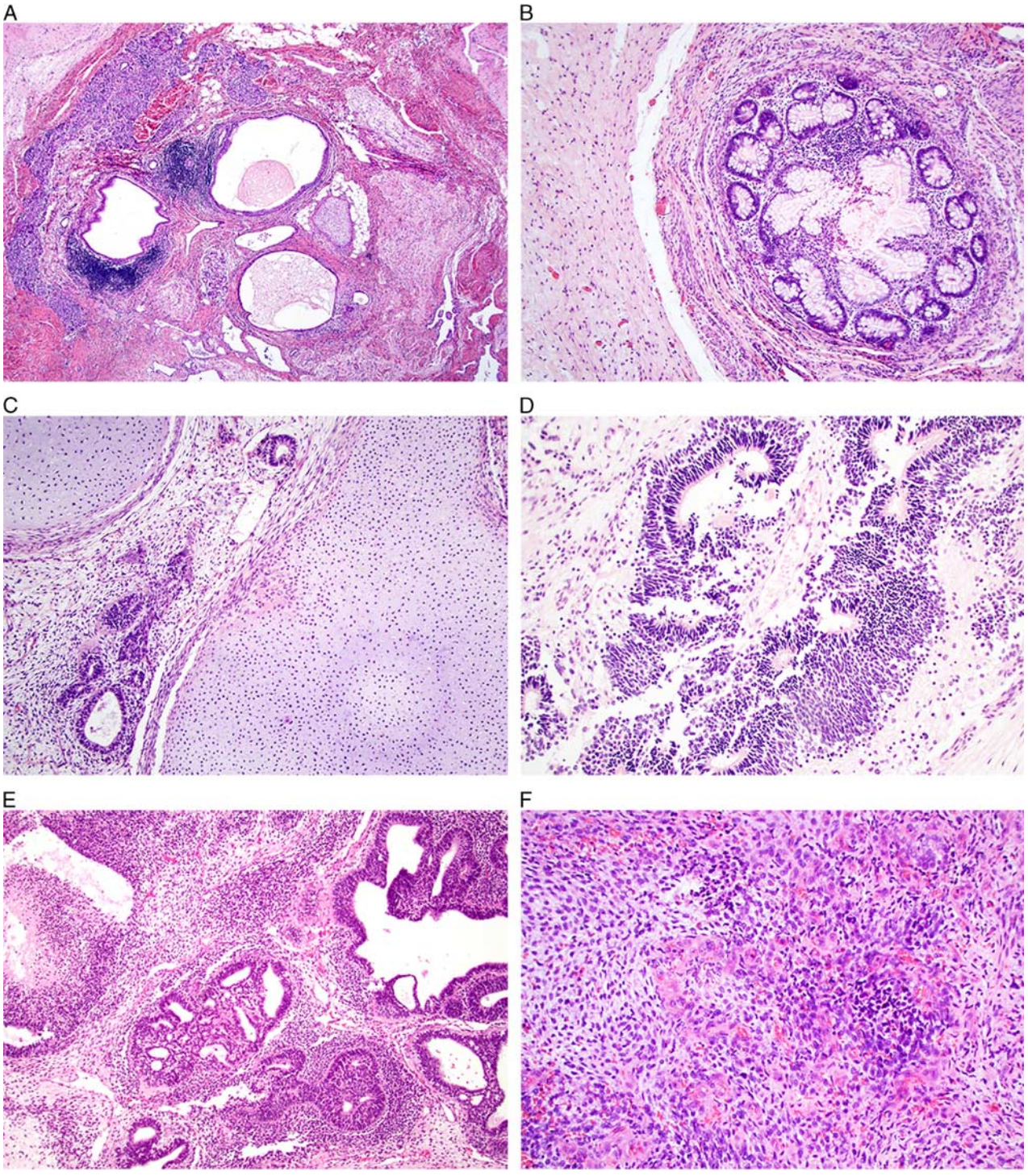

FIGURE 1. Benign teratomas were comprised of a diverse admixture of epithelial, mesenchymal, and neural tissues (A). A subset of benign teratomas displayed focal organoid growth with recapitulation of normal tissue architecture (B). While the single case of immature teratoma did demonstrate immature neural elements, they collectively occupied <1 low-power field in a background of more differentiated tissue (C). The immature neural tissue in the immature teratoma lacked significant cytologic atypia or necrosis (D). The malignant teratomas were composed of a mix of immature neural, spindled, and epithelial components (E). A subset of malignant teratomas showed a predominance of primitive spindled and epithelial elements rather than immature neural tissue (F).

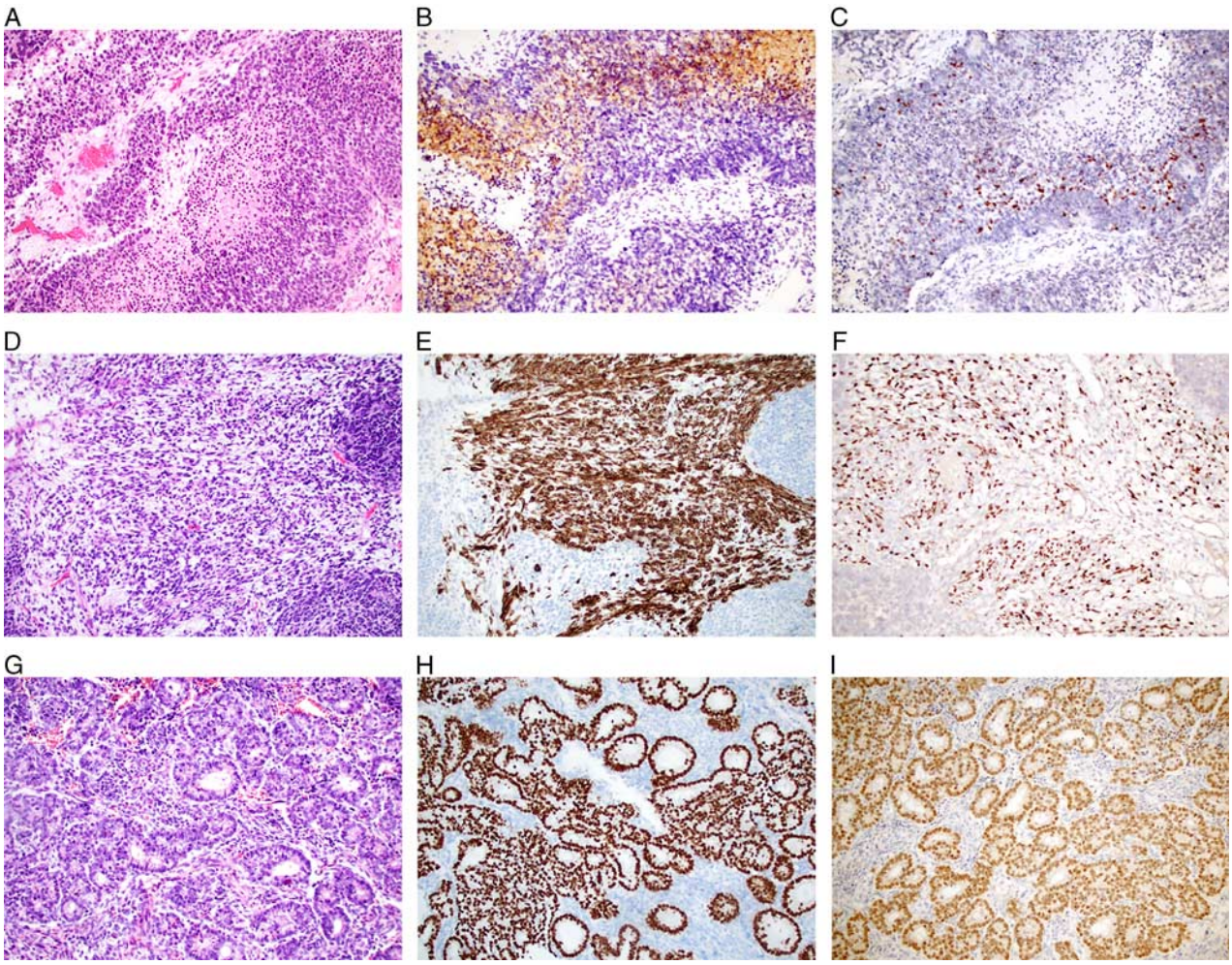

FIGURE 2. The malignant teratomas contained immature neural tissues with sheets and rosettes of hyperchromatic cells embedded in scant neuropil (A) and were positive for synaptophysin (B) and INSM1 (C). They also contained primitive spindle cell elements with variable amounts of eosinophilic cytoplasm (D) that showed rhabdomyoblastic differentiation with positivity for desmin (E) and myogenin (F). In addition, there were proliferations of bland cuboidal to columnar epithelial cells that formed glandular and cribriform structures (G) and were positive for TTF-1 (H) and PAX8 (I), consistent with thyroid follicular cell differentiation.

In this study, we report for the first time the presence of recurrent *DICER1* hotspot mutations in malignant thyroid teratomas. *DICER1* is a gene located on chromosome 14q32 that encodes an RNase IIIB enzyme that plays a key role in micro-MRA processing.[30] Pathologically, *DICER1* is best known for its role in the *DICER1* syndrome that predisposes patients to a constellation of tumors or tumor-like lesions including pleuropulmonary blastoma,[25,31] thyroid multinodular hyperplasia,[32] ovarian Sertoli-Leydig tumor,[18,24,29] embryonal rhabdomyosarcoma,[21] Wilms tumor,[23,33] nasal chondromesenchymal hamartoma,[28] well-differentiated thyroid carcinoma,[19,26,34] cystic nephroma,[21,35] ciliary body medulloepithelioma,[27,36] pineoblastoma,[37] pituitary blastoma,[38] anaplastic sarcoma of the kidney,[39] intracranial sarcoma,[40,41] and Mullerian adenosarcoma.[42] Rarely, somatic *DICER1* mutations can also give rise to the same tumors sporadically. Mutations in *DICER1* are thought to contribute to tumorigenesis through 2 distinct mechanisms. Hotspot mutations occur only in a critical group of nucleotides (E1705, E1813, D1709, D1810, and G1809) that affect metal-binding sites in the RNase IIIB enzyme domain and confer abnormal protein function with oncogenic potential even if the other allele is intact.[17,43,44] In contrast, nonhotspot mutations can occur at various sites in the *DICER1* gene and serve in a tumor suppressor capacity that requires biallelic inactivation for loss of function.[16,45] The 4 malignant teratomas in this study all harbored hotspot *DICER1* mutations, with additional nonhotspot loss of function mutations in 3 cases. Although germline DNA was not available for definitive assessment, the older patient age, lack of other *DICER1* syndrome-associated tumors, and variant allele fractions suggest that these malignant teratomas are of somatic origin and likely arose sporadically.

TABLE 2. NGS Results

Case No.	*DICER1* Hotspot (Variant Allele Frequency)	*DICER1* Nonhotspot (Variant Allele Frequency)	Other Mutations (Variant Allele Frequency)
1	p.E1705K (42.9%)	p.Y819fs (46.8%)	*BRD4* p.V350D (49.8%) *FGFR3* p.S779R (46.5%) *FBN2* p.A731G (39.2%) Chromosome 1p loss and 1q gain
2	p.E1813G (32.8%)	p.V448fs (39.5%)	*RET* p.R418X (35.4%) *NFKB2* p.Q164R (43.2%)
3	p.E1813Q (45.5%)	p.K868X (47.8%)	*KLF4* p.V262M (47.2%) *ARID2* p.H800D (50.2%) *NTRK3* p.R138W (47.8%) Chromosome 2 amplification
4	p.D1810H (9.4%)	None	*BCOR* p.Y755fs (14.7%) *BRD4* p.V1089I (49.0%) *HSP90AA1* p.G570A (43.1%) *FANCG* p.R485Q (48.1%)
5	None	None	*IGF1R* p.V1013fs (49.1%) *XRCC3* p.S327fs (44.8%)
6	None	None	*FLT3* p.S574N (48.4%) *KNL1* p.M788V (45.4%) *PARP4* p.A666S (9.1%) *RYR3* p.I3000V (43.5%)
7	None	p.K1844N (22.0%)	None
8	NP	NP	NP

NP indicates not performed due to inadequate DNA quality.

Despite their apparent extrasyndromic origin, our findings highlight several striking morphologic and immunohistochemical parallels between malignant thyroid gland teratomas and other *DICER1*-mutated tumors. While the broad spectrum of neoplasms that carry *DICER1* mutations includes benign and malignant entities with diverse histologic appearances, a few recurrent elements have been described in multiple tumor types. These include: (1) primitive neuroectodermal tissue (eg, ciliary body medulloepithelioma, pineoblastoma, pituitary blastoma), (2) rhabdomyoblastic spindle cell proliferations (eg, embryonal rhabdomyosarcoma, intracranial sarcoma), (3) bland epithelium homologous to the organ of origin (eg, pleuropulmonary blastoma, cystic nephroma, Mullerian adenosarcoma), and (4) heterologous elements including prominent islands of immature cartilage (eg, pleuropulomary blastoma, nasal chondromesenchymal hamartoma). In this study, we document the presence of parallel components in malignant thyroid gland teratomas. Of course, immature neural tissue was present in varying proportions in these tumors, with both synaptophysin and INSM1 providing immunohistochemical confirmation of its presence.[46,47] But malignant thyroid gland teratomas also consistently demonstrated primitive spindle cell elements with rhabdomyoblastic differentiation as evidenced by desmin and myogenin positivity, as well as bland immature epithelial proliferations of thyroid follicular cell origin with PAX8 and TTF-1 positivity. Although not present in every case, immature cartilage was also a prominent component of several malignant teratomas. These findings place malignant thyroid gland teratomas within the distinctive morphologic spectrum of *DICER1*-mutated neoplasia.

In contrast, malignant thyroid gland teratomas sharply differ from the differentiated thyroid lesions that have previously been associated with *DICER1* mutations. *DICER1* is well-established to play an important role in normal thyroid gland development, with contributions to orderly cell proliferation and differentiation.[48–51] Concordant with this function, the most common manifestation of *DICER1* syndrome in the thyroid gland is multinodular hyperplasia, with a markedly increased incidence of 75% in affected women and 17% in affected men by age 40.[32,34] Patients with germline *DICER1* mutations also have a 16- to 24-fold increased risk of well-differentiated thyroid cancers such as papillary or follicular thyroid carcinomas.[26,34] *DICER1* mutations have also been implicated in multinodular hyperplasia, papillary carcinoma, follicular carcinoma, and follicular adenoma in patients with no known syndromic association.[19,52–55] Only 2 *DICER1* mutations have previously been reported in cases of primitive or high-grade thyroid tumors: a *DICER1* E1813G hotspot mutation in another malignant thyroid gland teratoma[10] and a *DICER1* E1705K hotspot mutation in a malignant thyroid tumor with mixed epithelial and mesenchymal elements that was classified as a carcinosarcoma.[56] This study validates these findings in a larger cohort and provides the first documentation of a recurrent, primitive *DICER1*-associated phenotype in thyroid tumors.

Recognition of *DICER1* mutations in these primitive thyroid gland tumors also expands the pathologic boundaries of malignant thyroid gland teratoma as an entity. The World Health Organization (WHO) Classification of Endocrine Organ Tumours specifically requires thyroid teratomas to contain tissues from all 3 embryonal layers and defines malignancy based on the overgrowth of immature neural tissue.[1] For the purposes of this study, we expanded this classification to include tumors that contained only scant immature neural tissue but displayed a predominance of other primitive non-neural elements—a category that included the 50% of malignant teratomas in this cohort that showed a predominance of mesenchymal and epithelial elements. We identified *DICER1* hotspot mutations in all of the tumors that fit under this broader definition of malignant teratoma, regardless of their immature neural content. Furthermore, this spectrum of primitive thyroid tumors likely also encompasses tumors that do not strictly contain tissue from all 3 embryonal layers. One previously reported *DICER1*-mutated thyroid tumor noted above was originally classified as carcinosarcoma because it contained only epithelial and mesenchymal elements but appears to have had rhabdomyoblastic and thyroid follicular differentiation that are histologically and immunohistochemically similar to the malignant thyroid gland teratomas in this study.[56] A further expanded definition of malignant teratoma that requires only the presence of multilineage differentiation with a predominance of primitive hypercellular or overtly malignant elements may facilitate more comprehensive recognition of these aggressive tumors.

Finally, these findings further expand the distinction between benign/immature and malignant thyroid gland teratomas to the degree that they may merit a separate classification. While *DICER1* hotspot mutations were identified in all malignant teratomas in this study, they were absent in the small group of benign/immature thyroid gland teratomas we tested. These discrepant molecular findings parallel the vastly divergent clinical presentation and outcomes between these categories, suggesting that the different grades of teratoma represent distinct tumor types with unique causative mechanisms rather than a continuous clinicopathologic spectrum. Interestingly, a nonhotspot *DICER1* missense mutation was present in 1 case of benign teratoma in our series; this mutation has not previously been reported and its pathogenic significance is unclear. However, as discussed above, a nonhotspot mutation would not be expected to contribute to tumorigenesis without a synchronous mutation in the second allele. Although further exploration of the role of *DICER1* across the spectrum of thyroid gland teratomas in a larger patient cohort is certainly necessary, we propose that benign/immature and malignant teratomas might better be classified as separate entities. Recognition of 2 discrete categories of teratoma has some precedent in the testis, where benign pre–pubertal-type and malignant postpubertal-type teratomas are now regarded as clinically and molecularly separate entities[57–59]; a similar classification scheme that separates childhood from adult tumors could more accurately reflect the biology of teratomas in the thyroid gland. Moreover, in light of their similarities to other *DICER1*-mutated tumors and differences from teratomas at other anatomic sites, these findings also raise questions as to whether malignant thyroid teratomas should truly be classified as teratomas or even germ cell tumors at all. Similar *DICER1*-mutated teratoma-like lesions in the sacrococcygeal region have recently been reported as malignant teratoid neoplasms due to clinical, pathologic, and molecular differences from conventional teratomas.[60] Given their likely somatic origin and similarities to other *DICER1*-mutated tumors, parallel nomenclature that reclassifies malignant thyroid gland teratomas as malignant teratoid neoplasms should also be considered.

Overall, our findings demonstrate the consistent presence of *DICER1* hotspot mutations in malignant thyroid gland teratomas but not in benign/immature teratomas. These findings expand understanding of the pathogenic role of *DICER1* mutations in thyroid gland neoplasia and provide the first evidence of a recurrent role for *DICER1* in primitive thyroid gland tumors. The presence of variable immature neural, rhabdomyoblastic, and homologous epithelial elements in malignant thyroid gland teratomas illustrates histologic parallels to *DICER1*-mutated tumors in other organs and argues for a broader morphologic definition of this entity. Finally, these molecular findings further expand the differences between benign/immature and malignant thyroid gland teratomas and suggest that the latter category may not truly represent teratomas at all, supporting reclassification of these divergent grades as separate entities.

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