Ovarian Intermediate Trophoblastic Tumors

Genotyping Defines a Distinct Category of Nongestational Tumors of Germ Cell Type

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Abstract: Trophoblastic neoplasms involving the ovary are uncommon and include gestational tumors, which are either metastatic from the uterus or ectopic and nongestational tumors, which include those of germ cell type/origin and somatic tumors with trophoblastic differentiation; in all these types, most are pure choriocarcinoma. Intermediate trophoblastic tumors, which include placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT), are rare in the ovary, with most assumed to be gestational; this is the only category formally recognized in 2014 World Health Organization (WHO) classification, likely due to few well-documented nongestational examples. We report the clinicopathologic features of 6 ovarian intermediate trophoblastic tumors, including 3 PSTTs, 2 ETTs, and 1 ETT with choriocarcinomatous differentiation. DNA-based short tandem repeat genotyping identified 4 of these as nongestational (3 PSTTs and 1 ETT), as evidenced by sharing of alleles between tumor and normal tissue at all informative loci. Interestingly, all 3 of the nongestational PSTTs coexisted with mature cystic teratoma. The remaining 2 tumors (1 ETT and 1 ETT with some choriocarcinomatous differentiation) were gestational (likely ectopic due to lack of evidence of a uterine tumor), as evidenced by the presence of both maternal and novel/nonmaternal alleles at informative loci in tumor compared with normal tissue. It is important to recognize a distinct category of primary ovarian nongestational intermediate trophoblastic tumors of germ cell type/origin, including PSTT and ETT, in classification systems to guide clinical management, as gestational and nongestational tumors have different genetic origins and may require different therapy. Genotyping is useful for classification as nongestational versus gestational, particularly as traditional clinicopathologic findings cannot always predict the nature of a trophoblastic tumor.

Key Words: epithelioid trophoblastic tumor, molecular genotyping, nongestational trophoblastic tumor, ovarian germ cell tumor, placental site trophoblastic tumor

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In women, trophoblastic neoplasms include both gestational (common) and nongestational (uncommon) types. Those involving the ovary are rather uncommon and include gestational tumors that are metastatic from the uterus or ectopic, and nongestational tumors, which include those of germ cell type/origin and somatic tumors with trophoblastic differentiation[1,2]; in all these types, most are pure choriocarcinoma. Intermediate trophoblastic tumors, which include placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT), are rare in the ovary.[3–5] Most are assumed to be gestational tumors that are either metastatic from an occult uterine tumor or ectopic; this is the only category formally recognized in the World Health Organization (WHO) 2014 classification of ovarian tumors, likely due to few well-documented examples of nongestational tumors.[1]

The WHO 2016 classification of testicular germ cell tumors provides an expanded trophoblastic tumor category (obviously only comprised of nongestational tumors in males) which adds ETT and cystic trophoblastic tumor to the already included entities of choriocarcinoma and PSTT.[6–9] It seems reasonable to expect that, similar to the testis, some examples of PSTT and ETT in the ovary could also originate from germ cells. In fact, a unique consultation case in our institution of a primary ovarian PSTT in a 30-month-old girl who presented with isosexual precocious puberty of 1-month duration demonstrated that PSTT may rarely occur as a gonadal germ cell tumor in children.[4] Since that report, we have encountered several additional examples of ovarian PSTT and ETT, often associated with mature cystic teratoma, leading us to speculate that these intermediate trophoblastic tumors represent a distinct group of nongestational trophoblastic tumors of germ cell type/origin. We used DNA-based

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genotyping to analyze a series of ovarian intermediate trophoblastic tumors to determine their gestational versus nongestational nature. On the basis of the identification of a subset of nongestational tumors, we propose that the ovarian germ cell tumor classification should be modified to add a new category of nongestational intermediate trophoblastic tumors including PSTT and ETT.

MATERIALS AND METHODS

Case Selection

Cases were identified in the files of the authors' institutions (5 consultation cases from Johns Hopkins Hospital, 1 from Longgang Hospital, Zhejiang, China). Histologic sections of these cases were rereviewed by 2 pathologists (D.X. and B.M.R.) to confirm the diagnosis. The study was approved by the Institutional Review Board at the Johns Hopkins Hospital.

Immunohistochemistry

Immunohistochemical staining was performed in the Johns Hopkins Immunopathology Laboratory on formalin-fixed, paraffin-embedded (FFPE) tissue sections, using Ventana Benchmark automation and the Ultra View detection kit (Ventana Medical Systems, Tucson, AZ) as previously described.[10,11] Some stains were performed at the time of the original diagnosis and some were done for this study (depending on the availability of additional material for testing). Markers used included: hCG (mouse polyclonal; Ventana; prediluted), HSD3B1 (3C11-D4; Novus Biologicals, Littleton, CO; 1:2,000 dilution), GATA3 (L50-823; Biocare, Concord, CA; 1:100 dilution), hPL (rabbit polyclonal; Cell Marque, Rocklin, CA; prediluted), p63 (4a4; Biocare; prediluted), AE1/AE3 (pck-26, mouse polyclonal, Ventana; prediluted).

DNA Extraction

FFPE tumor tissues and corresponding normal tissues, identified by hematoxylin and eosin staining of adjacent sections were macrodissected (with tumor elements accounting for about $\geq 70\%$ of the section area), and genomic DNA was extracted using a QIAamp DNA FFPE Tissue Kit with an adapted protocol (Qiagen, Valencia, CA). Briefly, slides bearing paraffin-embedded tissue were baked at 68°C for 20 to 30 seconds; the tissue was deparaffinized 3 times with xylene, and residual xylene was removed by washing through serial dilutions of ethanol. The tumor tissue was separated from adjacent normal tissue and placed in a tube allowing for complete evaporation of residual ethanol. The tissue pellet was resuspended in Buffer ATL with added proteinase K. The rest of the procedure followed the manufacturer's instruction.

Molecular Genotyping

Genotyping was performed in the New York Medical College Laboratory using the GenePrint 24 System (Promega, Madison, WI). The GenePrint 24 System is a 24-locus, 5-color, multiplex system designed to generate a multilocus human DNA profile from a variety of human-derived biological sources. This system allows coamplification and fluorescent detection of 24 autosomal short tandem repeat (STR) loci plus Amelogenin for gender determination. We used 5 ng of template DNA which corresponds to ~600 diploid cells. The following polymerase chain reaction (PCR) cycling conditions were used as instructed by the manufacturer: after an initial denaturation of 1 minute at 96°C, 27 PCR cycles were performed at 94°C for 10 seconds, 59°C for 1 minute and 72°C for 30 seconds. After the reaction, an additional incubation step at 60°C for 10 minutes was added to enable full A-addition to the PCR products. Fragments were analyzed on the ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). A volume of 1 μL of the PCR reaction mix was added to 24 μL of deionized formamide (Sigma, St Louis, MO) and 1 μL of ROX-500 size standard (Applied Biosystems) or Internal Lane Standard 600 (Promega). Electrophoresis was performed using the Performance Optimized Polymer 4 (Applied Biosystems) with a 47 cm/50 μm capillary. Samples were injected over 3 seconds, and electrophoresis was performed according to the manufacturer's instructions. The Genescan software (Applied Biosystems) was used for size calling and quantification of peak areas. Because of the suboptimal quality of DNA extracted from FFPE tissue, foci that are >250 bp are not reliably amplified by PCR. Therefore, only ~15 foci and amelogenin are been able to be evaluated in this analysis.

RESULTS

Clinicopathologic features are provided in Table 1 and immunohistochemical analysis is summarized in Table 2. The 6 cases included 3 nongestational PSTTs, 1 nongestational ETT, 1 gestational ETT and 1 gestational ETT with choriocarcinomatous differentiation. All tumors presented as mass lesions in the ovary.

Primary Ovarian Nongestational Intermediate Trophoblastic Tumors

Three cases were consistent with nongestational PSTT associated with mature cystic teratoma. Case 1 was a 43-year-old woman who presented with an abdominal/pelvic mass and subsequently underwent bilateral salpingo-oophorectomy. The right ovary had a mass measuring 6 cm in maximal dimension, containing friable and cheesy material. The left ovary measured 11.0 cm in greatest dimension, demonstrated cystic and solid contents with yellow friable material and hair. A subsequent hysterectomy specimen was unremarkable. Case 2 was a 30-year-old woman with a 3 cm ovarian mass and increased serum β-hCG level of 1083 mIU/mL. The patient underwent right salpingo-oophorectomy and lymph node dissection. Case 3 has been reported previously[4] and we rereviewed the slides retrieved from our consultation archives. Briefly, the patient was a 30-month-old girl presented with isosexual precocious puberty and an elevated serum β-hCG level (37.5 mIU/mL). She underwent right salpingo-oophorectomy, revealing a 3.5 cm ovarian tumor with multiple complex hemorrhagic cysts.

Histologically, all 3 trophoblastic tumors displayed similar morphologic features, similar to uterine PSTTs. They were composed of loosely cohesive, predominantly mononucleated, large, round cells and polyhedral atypical cells with abundant eosinophilic, amphophilic, or clear cytoplasm, which in some areas replaced vessel walls; the cells displayed moderate to focally significant cytologic

TABLE 1. Clinicopathologic Features

Case No.	Age (y)	Clinical Presentation	β-hCG at Presentation (mIU/mL)	Specimen	Tumor Site and Size (cm)	Diagnosis	Origin
1	43	Abdominal/pelvic mass	Not tested	Bilateral ovaries and fallopian tubes	Right ovary: 6.0; left ovary: 11.0	Right ovary: PSTT; mature cystic teratoma; left ovary: mature cystic teratoma	Nongestational
2	30	Right ovarian mass with increased β-hCG level	1083	Bilateral ovaries and fallopian tubes	Right ovary: 3.0	PSTT; mature cystic teratoma; 1 para-aortic lymph node with metastasis	Nongestational
3	2.5 (30 mo)	Isosexual precocious puberty of 1 mo duration; right ovarian mass	37.5	Right ovary and fallopian tube	Right ovary: 3.6	PSTT; epidermoid cyst	Nongestational
4	39	Left ovarian mass with a second mass in the rectosigmoid colon	Not tested	Left ovary and rectosigmoid colon	Left ovary: 2.2; rectosigmoid colon: 6.5	ETT	Nongestational
5	36	Right ovarian mass	Not tested	Right ovary	Right ovary: 9.0	ETT	Gestational
6	55	Right ovarian mass	29,166	Uterus, cervix, bilateral ovaries, and fallopian tubes	Right ovary: 11.6	ETT with choriocarcinomatous differentiation	Gestational

atypia, with frequent convoluted, variably-sized hyperchromatic nuclei and occasional binucelated and trinucleated nuclei (Fig. 1A–C, Supplementary Figs. S1A–C, Supplemental Digital Content 1, http://links.lww.com/PAS/A872 and Supplementary Figs. S2A–E, Supplemental Digital Content 2, http://links.lww.com/PAS/A873). The tumors had an infiltrative growth of aggregates to sheets of cells into a mature cystic teratoma (case 1 and case 2) and infiltrated between primordial follicles (case 3). In particular, on review of the slides for case 3, a minor component of a small epidermoid cyst was identified (Supplementary Fig. S2A, Supplemental Digital Content 2, http://links.lww.com/PAS/A873; not mentioned in the prior report).

Immunohistochemically, the tumor cells in all 3 cases were diffusely immunoreactive for hPL (Fig. 1D, Supplementary Fig. S1D, Supplemental Digital Content 1, http://links.lww.com/PAS/A872 and Supplementary Fig. S2F, Supplemental Digital Content 2, http://links.lww.com/PAS/A873), focally positive for hCG (Fig. 1E, Supplementary Fig. S1E, Supplemental Digital Content 1, http://links.lww.com/PAS/A872 and Supplementary Fig. S2I, Supplemental Digital Content 2, http://links.lww.com/PAS/A873) and had only rare p63-positive cells (Fig. 1F, Supplementary Fig. S1F, Supplemental Digital Content 1, http://links.lww.com/PAS/A872). The staining pattern of hPL in case 3 was described as focal in the previous report but slide review disclosed diffuse expression. The tumor cells were also diffusely positive for HLA-G (cases 2 and 3; Supplementary Fig. S2G, Supplemental Digital Content 2, http://links.lww.com/PAS/A873), Gata3 (case 2), AE1/AE3 (case 3, Supplementary Fig. S2H, Supplemental Digital Content 2, http://links.lww.com/PAS/A873), and Mel-CAM (case 3).

Genotyping for case 1 was performed to compare the PSTT (right ovary), mature cystic teratoma (left ovary [insufficient pure tumor tissue from right ovarian teratoma component for analysis]), and normal maternal tissue (left fallopian tube). STR analysis demonstrated that the DNA pattern from the PSTT was similar to that of maternal tissue—only shared alleles without any novel/nonmaternal alleles, with heterozygous/biallelic loci having near equal allele ratios—consistent with a nongestational tumor (Fig. 2). This heterozygous/biallelic pattern is consistent with what has been reported for some ovarian teratomas and

TABLE 2. Immunohistochemical Results

Case No.	hCG	hPL	p63	HSD3B1	GATA3	AE1/AE3	Diagnosis (Origin)
1	Focally positive	Positive (diffuse)	Rare positive cells	Positive (diffuse)	NA	NA	PSTT (nongestational)
2	Focally positive	Positive (diffuse)	Rare positive cells	NA	Positive (diffuse)	NA	PSTT (nongestational)
3	Focally positive	Positive (diffuse)	NA	NA	NA	Positive (diffuse)	PSTT (nongestational)
4	Focally positive	Rare positive cells	Focally positive	Positive (diffuse)	Positive (diffuse)	Positive (diffuse)	ETT (nongestational)
5	Focally positive	Rare positive cells	Positive (diffuse)	Positive (diffuse)	Positive (diffuse)	NA	ETT (gestational)
6	Positive (diffuse)	Negative	Positive (diffuse)	Positive (diffuse)	Positive (diffuse)	Positive (diffuse)	ETT with choriocarcinomatous differentiation (gestational)

NA indicates not available.

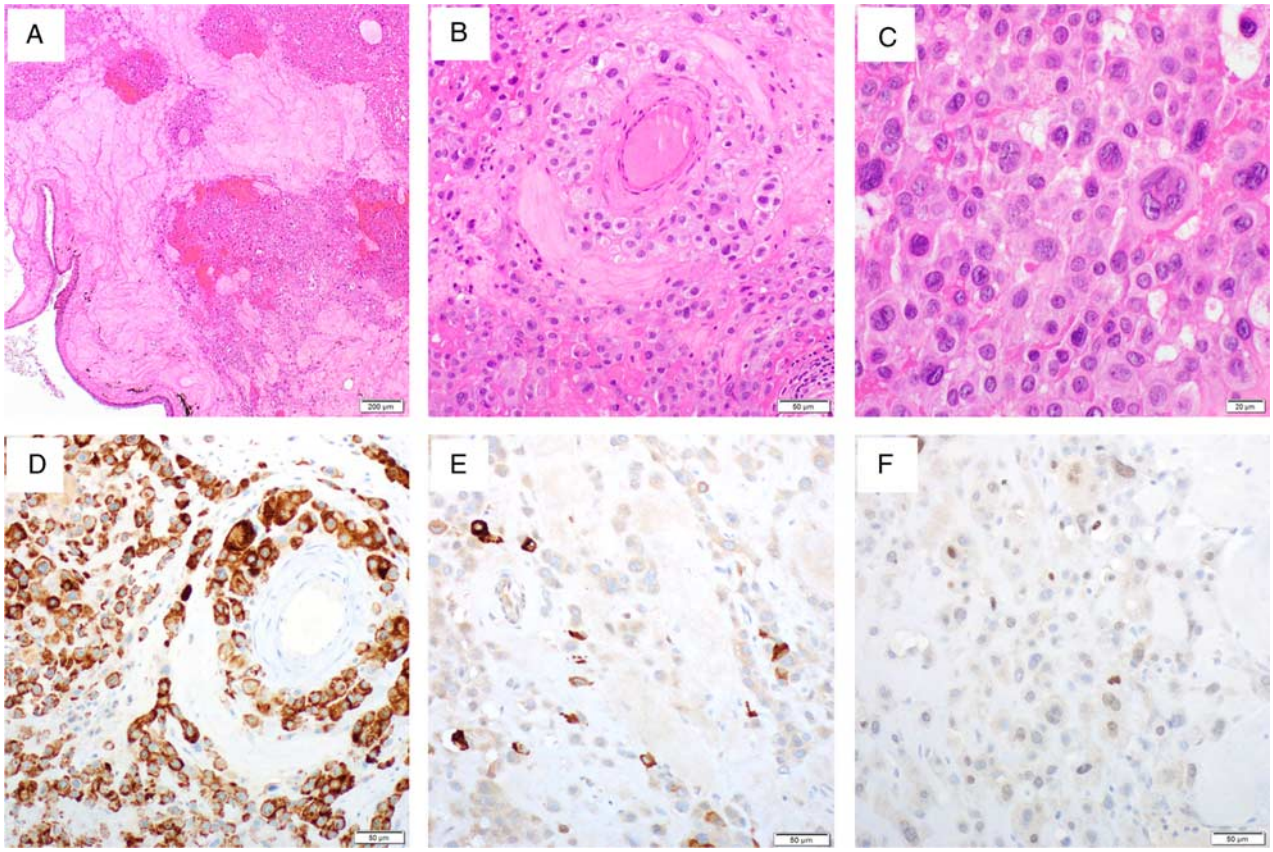

FIGURE 1. Nongestational PSTT in the ovary (case 1) with mature cystic teratoma (A). Some areas show replacement of vascular walls by tumor cells and deposition of fibrinoid material (B). The tumor cells display abundant eosinophilic, amphophilic, or clear cytoplasm, and moderate to significant cytologic atypia with frequently convoluted nuclei and hyperchromasia (C). The intermediate trophoblastic cells are diffusely immunoreactive for hPL (D) and show a focal expression of hCG (E) and p63 (F).

consistent with neoplastic transformation of a premeiotic diploid germ cell or as a result of meiosis I failure.[12] The DNA pattern from the mature cystic teratoma in the left ovary also matched the maternal DNA pattern (no novel/nonmaternal alleles), also consistent with a nongestational tumor as expected for a teratoma. The allele ratios were consistent with a homozygous allele pattern when factoring out contaminating maternal DNA (Fig. 2). This pattern is consistent with what has been reported for the majority of ovarian teratomas and is consistent with the neoplastic transformation of germ cells after completion of meiosis I with failure of meiosis II.[12] By report, STR analysis for case 2 performed at an outside laboratory revealed a homozygous pattern in tumor DNA with all alleles matched to the maternal tissue consistent with a nongestational tumor. Repeat genotyping performed by our team on the tumor demonstrated a pattern similar to the teratoma result in case 1 (data not shown) but normal tissue was not available for comparative testing (international case for which only tumor slides could be obtained). Although material was not available for genotyping for case 3, we classified the tumor as a nongestational PSTT based on the morphology, immunoprofile on review, as well as the patient's age (30-mo-old).

Follow-up data with limited information was available for all 3 cases. For case 1, the patient's serum β-hCG level was <2 mIU/mL at 16 months and there was no evidence of disease at 39 months of follow-up. As metastatic tumor was present in 1 para-aortic lymph node, the patient of case 2 underwent chemotherapy and had no evidence of disease during 10 months of follow-up with a serum β-hCG level of 0.16 mIU/mL. The child of case 3 had no evidence of disease recurrence, with undetectable serum β-hCG level during 24 months of follow-up. The information as to whether the patients of case 1 and case 3 underwent chemotherapy was not available.

One case (case 4) was consistent with nongestational ETT. The patient was a 39-year-old woman who was originally seen for a rectosigmoid colon mass with bowel perforation when an ovarian mass was found. Both tumors were similar, characterized by intermixed nodular growth of medium-sized epithelioid tumor cells arranged in nests and cords with geographic necrosis (Figs. 3A, B). The tumor cells were relatively uniform, with abundant eosinophilic cytoplasm and round, hyperchromatic nuclei and occasionally prominent nucleoli (Fig. 3C). Nuclear atypia was moderate but scattered large, pleomorphic tumor cells were seen. Extracellular eosinophilic hyaline-like material

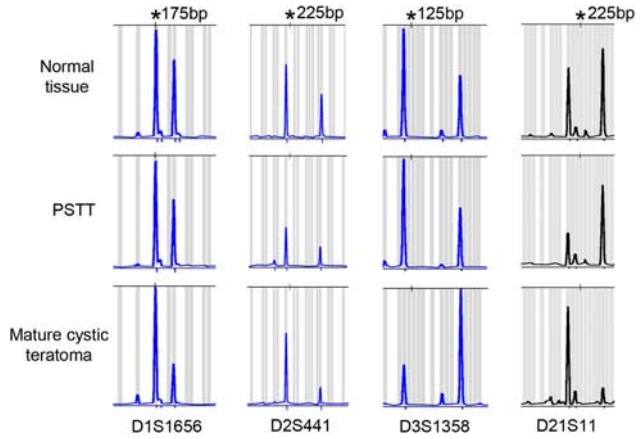

FIGURE 2. Genotyping of case 1 demonstrates that the DNA pattern from the PSTT matches the maternal (normal tissue) DNA pattern, without any novel/nonmaternal alleles, consistent with a nongestational tumor. The DNA pattern from the mature cystic teratoma also shares all alleles with the maternal pattern. On the basis of the allele ratios (1 significantly taller peak at each locus), this pattern is most consistent with a mixture of homozygous tumor DNA and biallelic maternal DNA in which the tall peak represents a single homozygous allele predominantly from tumor, with a portion of this contributed by the contaminating maternal DNA, and the smaller peak represents another contaminating purely maternal allele not present in the tumor. *Tickmark location of the size of PCR product.

was readily recognized. The tumor infiltrated the bowel wall and extended into the mucosa (Fig. 3D). Some areas of the tumor had a variant appearance, displaying a mixture of epithelioid and spindle cells with increased atypia and occasional multinucleated cells (Figs. 3E, F). In both components (the pure epithelioid and mixed epithelioid and spindle cell components), tumor cells were extensively positive for HSD3B1 (Fig. 3G), GATA3, and Mel-CAM, with a focal expression of p63 (Fig. 3H) and hCG (Fig. 3I) as well as rare hPL-positive cells. The diminished p63 staining was seen in both the epithelioid and spindle cell components. The purely epithelioid component was consistent with an ETT and based on the blending of the components and the similar immunoprofile, we interpreted the spindle cell component as a variant of ETT. Teratoma was not identified in the available sections. STR analysis demonstrated that the DNA pattern from the ETT was similar to that of maternal tissue—only shared alleles without any novel/nonmaternal alleles, with heterozygous/biallelic loci having near equal allele ratios—consistent with a nongestational tumor (Fig. 4). Similar to the tumor in case 1, this pattern is consistent with neoplastic transformation of a premeiotic diploid germ cell or as a result of meiosis I failure. Follow-up information was not available.

Primary Ovarian Gestational Intermediate Trophoblastic Tumors

There were 2 primary ovarian gestational intermediate trophoblastic tumors identified, including 1 ETT and 1 tumor classified as ETT with some choriocarcinomatous differentiation.

Case 5 was a 36-year-old woman with a 9 cm right ovarian tumor who underwent right salpingo-oophorectomy. The tumor was characterized by cellular islands with an eosinophilic hyaline matrix surrounded by geographic necrosis (Fig. 5A) and areas with calcifications (Fig. 5B). Predominantly monotonous epithelioid tumor cells with eosinophilic cytoplasm were arranged in a slightly nested pattern in cellular areas (Fig. 5C). The medium-sized tumor cells were relatively uniform with a moderate amount of finely granular cytoplasm, distinct cell membranes and round nuclei with distinct small nucleoli. Nuclear atypia was moderate and the mitotic index was 4 mitotic figures per 10 HPFs. The tumor cells were diffusely positive for HSD3B1, GATA3, and p63 (Fig. 5D), with only scattered cells positive for hCG (Fig. 5E) and hPL (Fig. 5F). The morphology and immunoprofile were consistent with an ETT. STR analysis demonstrated a biparental allelic pattern, with the presence of both maternal and novel/nonmaternal alleles, supporting interpretation as a gestational tumor (Fig. 6). There was no information available regarding the status of the endometrium/uterus, so a metastatic versus ectopic nature could not be definitively determined, but, given the lack of clinical evidence of a uterine tumor, it is possible that the ovarian tumor was ectopic. No follow-up information could be obtained.

Case 6 was a 55-year-old woman with an 11.6 cm right ovarian mass and serum β-hCG of 29,166 mIU/mL. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed, revealing a multilobulated solid mass with extensive necrosis in the right ovary but an unremarkable uterus. Islands of the highly cellular tumors were intimately associated with regions of necrosis (Supplementary Figs. S3A, B, Supplemental Digital Content 3, http://links.lww.com/PAS/A874). Monotonous atypical epithelioid tumor cells had rounded nuclei and scant pale cytoplasm (Supplementary Fig. S3C, Supplemental Digital Content 3, http://links.lww.com/PAS/A874). In the central regions of some tumor nests, occasional partially necrotic/degenerated tumor cells with multiple nuclei and abundant eosinophilic cytoplasm consistent with syncytiotrophoblastic cells were identified. The tumor cells were diffusely immunoreactive for hCG (Supplementary Fig. S3D, Supplemental Digital Content 3, http://links.lww.com/PAS/A874), p63 (Supplementary Fig. S3E, Supplemental Digital Content 3, http://links.lww.com/PAS/A874), HSD3B1, and GATA3 and negative for hPL (Supplementary Fig. S3F, Supplemental Digital Content 3, http://links.lww.com/PAS/A874). Although the morphology and diffuse p63 expression were most in keeping with an ETT, the focal syncytiotrophoblastic cells, diffuse hCG expression, and significantly increased serum β-hCG levels favored interpretation as a predominantly monomorphic form of malignant trophoblastic neoplasm dominated by features of ETT but with evidence of choriocarcinomatous differentiation. STR analysis demonstrated an androgenetic allelic pattern (only homozygous novel/nonmaternal alleles at all fully informative loci), supporting interpretation as a gestational tumor,

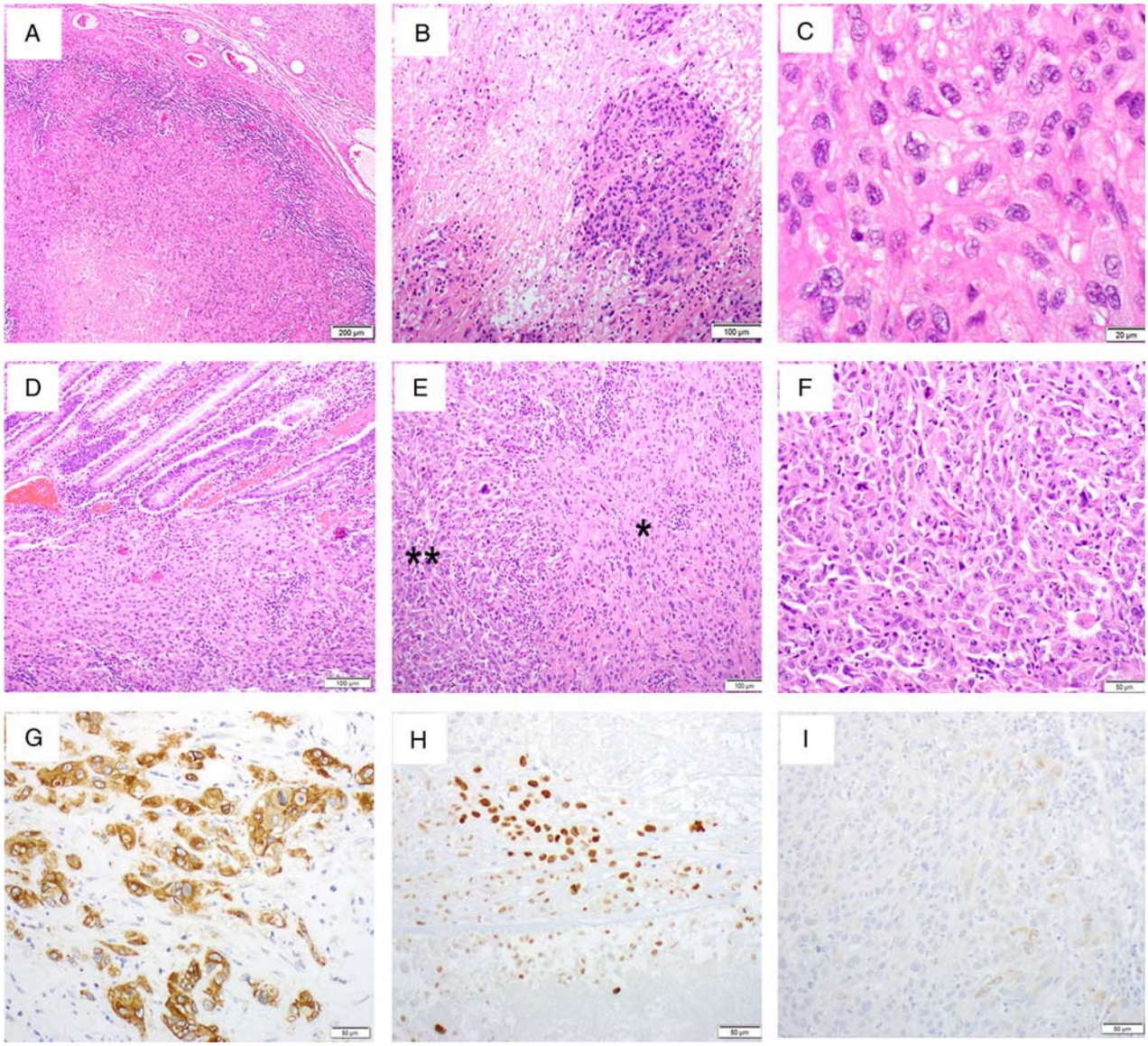

FIGURE 3. Nongestational ETT in the ovary (case 4). The sections demonstrate intermixed nodular growth of medium-sized tumor cells arranged in nests and cords (A) with geographic necrosis (B). The tumor cells are relatively uniform, with abundant eosinophilic cytoplasm, round, hyperchromatic nuclei, and occasionally prominent nucleoli (C). Nuclear atypia is moderate but scattered large, pleomorphic tumor cells are seen. Extracellular eosinophilic hyaline-like material is present. The tumor demonstrates bowel involvement (D). Some areas show typical features of ETT (E, *) transitioning to a spindle cell component (E, **) with increased atypia and occasional multinucleated cells (F). Epithelioid component demonstrates diffuse expression of HSD3B1 (G), focal expression of p63 (H) and limited weak expression of hCG (I).

consistent with an ectopic tumor based on the gestational nature and lack of a uterine tumor (Supplementary Fig. S4, Supplemental Digital Content 4, http://links.lww.com/PAS/A875). Follow-up information was not available.

DISCUSSION

Our case series characterizes an uncommon set of primary ovarian intermediate trophoblastic tumors, which include PSTTs and ETTs, with tumors of both gestational and nongestational types/origins established by DNA-based genotyping. The nongestational forms of PSTT and ETT presumably arise from germ cells, which are speculated to undergo neoplastic transformation into these intermediate trophoblastic tumors, likely as a variation of the process by which nongestational choriocarcinoma arises in the ovary. This idea is supported by the presence of intimately associated mature cystic teratoma in 3 of 4 nongestational tumors. Although rare, it is reasonable to expect that such tumors can occur in the ovary, as the occurrence of nongestational PSTT and ETT of germ cell origin in the testis is well-documented.[6,13–16] In particular, and quite similar to our case of PSTT with teratoma on

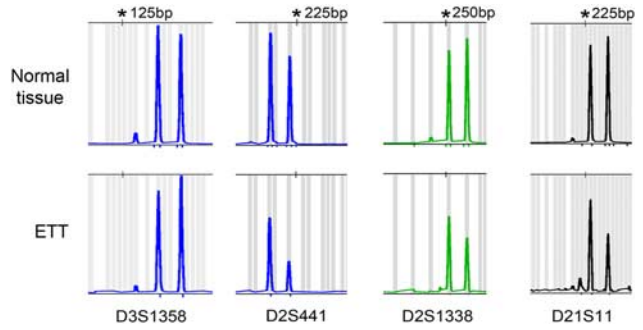

FIGURE 4. Genotyping of case 4 demonstrates that the DNA pattern from the ETT matches the maternal (normal tissue) DNA pattern, without any novel/nonmaternal alleles, consistent with a nongestational tumor. *Tickmark location of the size of PCR product.

a 30-month-old girl, a nongestational PSTT (1.5 cm) with adjacent small epidermoid cyst was previously reported in the right testis of a 16-month-old boy.[17] In addition, nongestational PSTT and ETT have been reported in nonhuman primates.[18–20] Although teratomatous elements were not identified in the nongestational ETT in our series, it is possible that such elements were not found due to sampling issues or possible overgrowth of a teratomatous component. Regardless, genotyping established that the tumor was nongestational.

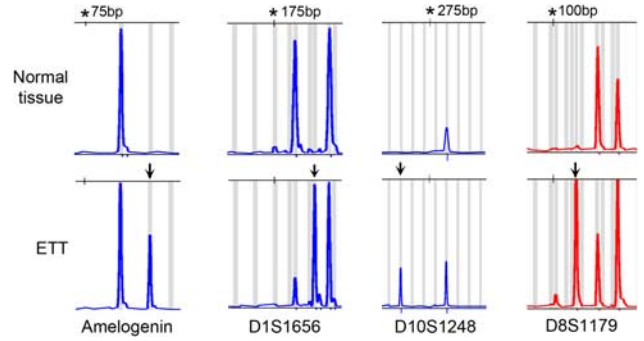

FIGURE 6. Genotyping of case 5 demonstrates a biparental allelic pattern, with the presence of both maternal and novel/nonmaternal alleles (arrows, including a Y chromosome [shorter peak at Amelogenin locus]; minor peaks in some loci are consistent with maternal contamination and/or suboptimal DNA template quality), consistent with a gestational tumor. *Tickmark location of the size of PCR product.

Among our cases, 3 nongestational PSTTs and 1 gestational ETT displayed typical morphologic features of these tumors. Two tumors had some variant features. These included 1 nongestational ETT in which the tumor had areas with typical features of ETT as well as other areas with a mixture of epithelioid and spindled cells with increased atypia and occasional multinucleated giant cells. Both of

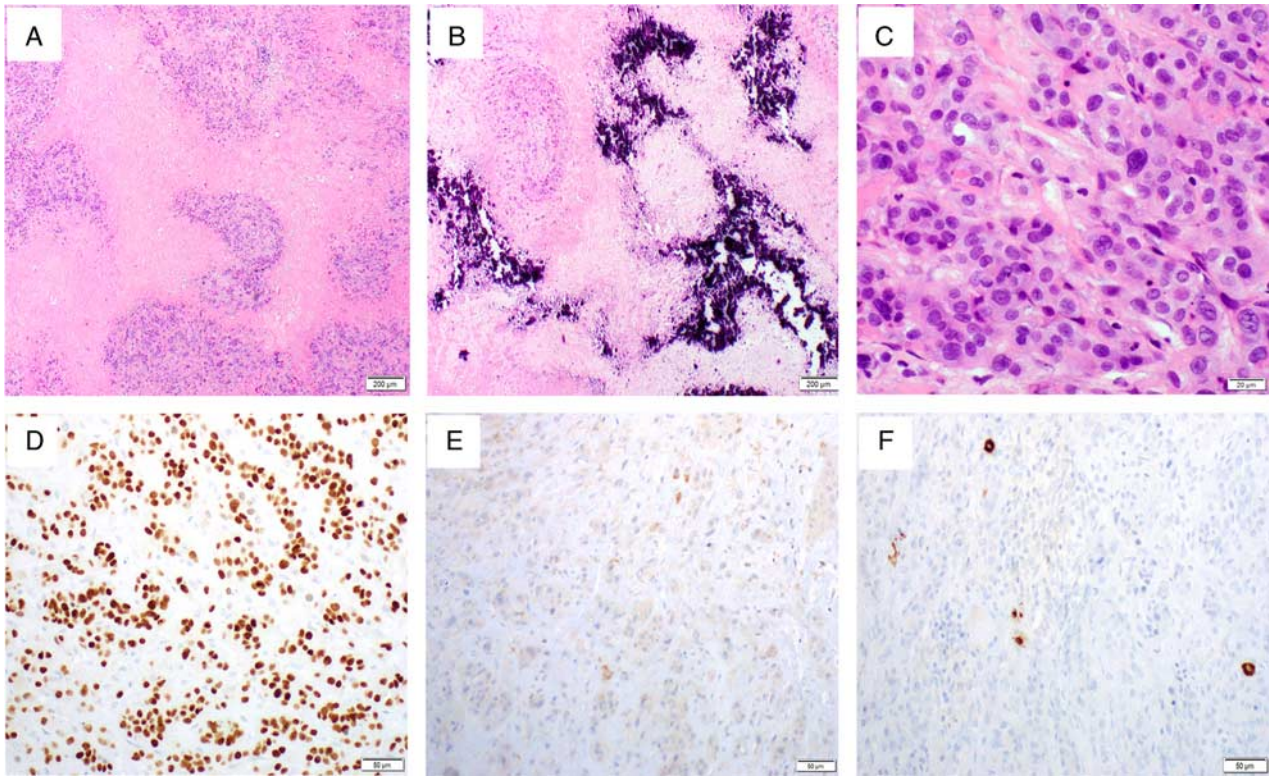

FIGURE 5. Gestational ETT in the ovary (case 5). The tumor is characterized by cellular islands with an eosinophilic hyaline matrix surrounded by geographic necrosis (A) and areas with calcifications (B). Predominantly monotonous epithelioid tumor cells with eosinophilic cytoplasm are arranged in a slightly nested pattern in cellular areas (C). The tumor cells are diffusely positive for p63 (D), and only occasional cells express hCG (E) and hPL (F).

these components shared the same immunoprofile, supporting interpretation as a variant pattern of ETT, probably with some degree of higher-grade malignant transformation. This tumor had less p63 expression than usually encountered in ETT, which is typically diffusely positive,[21] but the epithelioid component was morphologically characteristic. The other tumor with variant morphology was characterized by highly cellular mononucleate trophoblast with geographic necrosis, consistent with a malignant form of ETT, but some tumor islands contained partially necrotic/degenerating multinucleated syncytiotrophoblastic cells, indicating some choriocarcinomatous differentiation. Thus, this tumor was interpreted as a malignant form of ETT with some choriocarcinomatous differentiation rather than as a monomorphic form of pure choriocarcinoma based on the dominant characteristic morphology.

The data in our series is too limited to provide meaningful insights into the behavior and prognosis of these tumors. However, 2 nongestational tumors, 1 PSTT, and 1 ETT, demonstrated extra-ovarian spread manifested as lymph node metastasis in the former and bowel invasion in the latter. Thus, there is some evidence that these tumors have the potential to behave in an aggressive fashion, however, data on behavior of nongestational intermediate (nonchoriocarcinomatous) trophoblastic tumors are limited. Interestingly, nonchoriocarcinomatous trophoblastic tumors of the testis, including PSTT and ETT, appear to have a less aggressive clinical course, illustrated by the reporting of 7 patients with these tumors who were alive and well on follow-up (median, 39 mo), whereas only 1 patient with a hybrid tumor (mixture of adenocarcinoma and unclassified trophoblastic tumor) died of liver metastases at 2 years.[6] We suspect the latter case represents a somatic carcinoma with trophoblastic differentiation, which would be expected to have aggressive behavior. This is based on our previous study and the published literature demonstrating that the majority of somatic tumors with trophoblastic/choriocarcinomatous differentiation affect postmenopausal women and have a poor clinical course—the median survival period is usually <1 year.[2,22–24] Although nongestational intermediate trophoblastic tumors in the ovary may very well not behave as aggressive as these somatic tumors with trophoblastic/choriocarcinomatous differentiation, we have limited data on which to base prognosis because our cases represent rare tumors and are mostly encountered as consultation cases for which it is difficult to get complete follow-up data. Analysis of more of these uncommon intermediate trophoblastic tumors in nonuterine sites, with more extensive follow-up, would be desirable. Determination of their gestational versus nongestational nature is advocated so that any differences in behavior and different therapeutic approaches can be evaluated.

Extrauterine intermediate trophoblastic tumors have been reported in the ovary, fallopian tube, broad ligament, and lung.[3–5,25] In women of reproductive age, it is more likely that these tumors are gestational, presumably arising from an ectopic pregnancy or as a metastasis from an occult uterine gestational trophoblastic tumor. However, the current series and prior reports demonstrate that tumors in nonuterine sites, especially when there is a pathologically unremarkable endometrial or uterine specimen available, can be nongestational. Given that DNA-based genotyping provides a powerful tool to evaluate whether a trophoblastic tumor is gestational or nongestational,[26–28] we advocate for this analysis of trophoblastic tumors, particularly when arising in unusual sites or in unusual clinical scenarios. This is important because, similar to choriocarcinomas,[26,29–33] the clinical findings do not always predict the gestational versus nongestational nature of a trophoblastic tumor and there may be differences in behavior between gestational and nongestational intermediate trophoblastic tumors, requiring different therapeutic approaches. In this series, the 2 ovarian gestational intermediate trophoblastic tumors are speculated to have originated from ectopic pregnancies. Despite the age of 55 years in case 6, the tumor was genotyping-proven as gestational and the uterus was unremarkable. In case 5, metastasis from an occult uterine tumor could not be excluded, but there was no evidence provided to confirm that either.

Genotyping confirmed that a distinct category of primary ovarian nongestational intermediate trophoblastic tumors, including PSTT and ETT, exists in addition to the gestational forms of these tumors. We, therefore, propose 2 pathways by which these tumors can arise as primary ovarian tumors (Fig. 7). These include via an ectopic pregnancy and from ovarian germ cells. It is reasonable to propose that neoplastic cytotrophoblastic stem cells in an ectopic conception, similar to what is accepted for the development of these tumors in the uterus, differentiate into implantation-type intermediate trophoblastic cells to give rise to PSTT and into chorionic-type intermediate trophoblastic cells to give rise to ETT.[34,35] However, the pathogenesis of nongestational PSTT and ETT in the ovary remains largely unknown. In the testis, it is postulated that the most primitive type of trophoblastic tumor, choriocarcinoma, is able to progress to form a spectrum of trophoblastic tumors including PSTT and ETT.[6] Theoretically, it is possible that intermediate trophoblastic differentiation in a somatic tumor, similar to choriocarcinomatous differentiation in somatic carcinomas,[2] could also occur as another pathway, but this observation has not been reported in the literature.

Genotyping not only provides information about the gestational versus nongestational nature of a trophoblastic tumor but also provides some insight into potential pathogenetic pathways that contribute to tumorigenesis. A recent study showed that both immature and mature teratomas can have a homozygous or a heterozygous genome,[12] providing some evidence for the stage in the meiotic process at which the tumor likely developed. Interestingly, genotyping of 2 tumors in case 1 revealed different allelic patterns: the mature cystic teratoma in the left ovary demonstrated a homozygous pattern, suggesting a tumor that arose from a germ cell after having undergone meiosis I, whereas the PSTT in the right ovary showed a heterozygous/biallelic pattern, suggesting a tumor that arose from a premeiotic germ cell or in the setting of meiosis I failure. The teratoma coexisting with the PSTT could not be genotyped to determine whether it shared the DNA pattern with the PSTT and whether the PSTT was

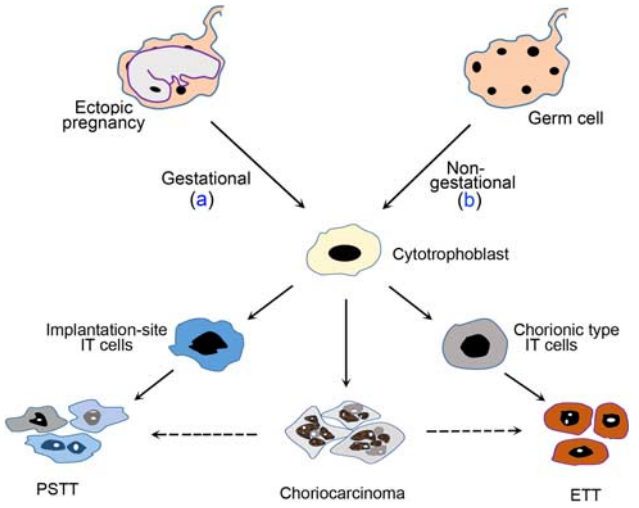

FIGURE 7. Model for primary ovarian trophoblastic tumor development. A, The gestational trophoblastic tumor is related to ectopic pregnancy and originates from cytotrophoblastic stem cells in either an ectopic molar pregnancy or an ectopic nonmolar conception. B, The nongestational trophoblastic tumors can be of germ cell origin. Two possible pathogenic pathways are proposed in this latter scenario: (1) germ cells differentiate into cytotrophoblastic stem cells which subsequently transform into the most primitive form of trophoblastic tumor—choriocarcinoma—which can differentiate to give rise to a spectrum of trophoblastic tumor types including PSTT and ETT (middle pathway); (2) germ cells differentiate into cytotrophoblastic stem cells which subsequently differentiate into different types of intermediate trophoblastic (IT) cells, with those of implantation site-type giving rise to PSTT (left pathway) and those of chorionic-type giving rise to ETT (right pathway).

therefore likely arising from it, but as teratomas can have a heterozygous/biallelic pattern the result for the PSTT is not inconsistent with origin from the associated teratoma. Genotyping of the PSTT in case 2 revealed a pattern that was similar to the mature cystic teratoma in case 1, indicating this tumor had arisen from a cell that had undergone meiosis I. The observation of these 2 different patterns for 2 nongestational PSTTs occurring with teratomas, which mirror the patterns encountered in pure teratomas, provides support for the germ cell origin of these tumors and indicates that ovarian nongestational intermediate trophoblastic tumors can also arise at different stages in the meiotic process. Analysis of more cases is required to confirm these observations and determine whether there is any biological or clinical significance to these different origins.

In summary, the current study characterizes a distinct group of primary ovarian intermediate trophoblastic tumors and identifies a nongestational set consistent with germ cell type/origin, including PSTT and ETT. Genotyping is recommended for assessment as nongestational versus gestational, particularly since traditional clinicopathologic findings cannot always predict the nature of a trophoblastic tumor. This category should be recognized in classification systems to guide clinical management, as gestational and nongestational tumors have different genetic origins/pathogenesis and may require different therapy.

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