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ARTICLE

Precision genotyping diagnosis of lung tumors with trophoblastic morphology in young women

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Abstract

Trophoblastic differentiation has been previously described in somatic carcinomas at different primary sites, including the lung. Lung carcinomas with trophoblastic morphology presenting in women during the reproductive years pose a unique diagnostic challenge due to their overlapping microscopical and immunophenotypical features with metastatic choriocarcinoma of gestational origin. Distinction between the two entities is paramount as they require different chemotherapeutic regimens and have a markedly different prognostic outlook. Here we report a series of three female patients (ages 37–48 years) presenting with lung masses. Two of the three patients were noted to have elevated serum beta-hCG levels at the time of their presentation, while serum beta-hCG was not evaluated preoperatively in the third patient. None of them had a clinical history of molar pregnancy or gestational trophoblastic neoplasia. Core biopsies of the lung masses were performed in two patients and one patient underwent a wedge resection, showing poorly differentiated carcinoma in all cases with scattered multinucleated giant cells, hemorrhage, and necrosis. Beta-hCG immunostain was performed in two cases and showed diffuse immunoreactivity. Clinical history and imaging studies were not conclusive in any of the cases to rule out a gestational origin. Short tandem repeat genotyping analysis was performed to compare the allelic patterns between tumor and normal tissues and revealed identical profiles in one case, consistent with somatic origin, and unique paternal alleles in two cases, confirming metastatic gestational choriocarcinoma. The patient with primary somatic lung carcinoma died of disease within 15 months despite chemotherapy, while both patients with gestational choriocarcinoma responded well to chemotherapy and are alive without evidence of disease. Our cases illustrate the diagnostic pitfalls of lung tumors with trophoblastic differentiation in young women. Genotyping analysis offers precise diagnostic distinction between primary lung carcinoma and gestational choriocarcinoma with major therapeutic and prognostic implications for the patients.

Introduction

Trophoblastic differentiation in neoplasms may arise through three fundamentally different pathogenetic mechanisms. Gestational trophoblastic tumors—gestational choriocarcinoma, epithelioid trophoblastic tumor, and placental site trophoblastic tumor—develop from an antecedent gestational event, a term pregnancy, abortion, or a hydatidiform mole, and thus have unique paternal genetic contribution [1–5]. Germ cell tumors of the ovary, testis, or rarely extragonadal sites may be histologically entirely or partially composed of trophoblastic elements (pure or mixed choriocarcinomas), are not associated with a prior gestation and lack paternal genetic material [6, 7]. In addition, focal trophoblastic differentiation, morphologically presenting as choriocarcinoma or as scattered syncytiotrophoblastic giant cells, has been reported in various somatic carcinomas, including endometrial adenocarcinoma, ovarian and cervical clear cell carcinoma, urothelial carcinoma of the bladder, and lung carcinoma, and is thought to arise from the somatic component through clonal progression [8–16].

Diagnostic distinction based on the histogenesis of these tumors—gestational, germ cell, or somatic—is paramount due to their marked differences in prognosis and clinical treatment protocols. In most cases the patient's clinical presentation, history, and other clinicopathological parameters are sufficient to determine the

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specific pathogenetic entity. However, the differential diagnosis of trophoblastic lung tumors in women during their reproductive years can be difficult to resolve in the absence of history of a molar gestation, uterine bleeding, or a pelvic mass lesion. Molecular genotyping is now considered part of the routine diagnostic algorithm for molar gestations in many academic centers, and can also be a powerful ancillary tool in this setting to confirm gestational origin of the lung tumor by identifying unique paternal alleles.

Materials and methods

Three cases were identified prospectively in our department within a 2-year period with a differential diagnosis including a primary lung carcinoma and a metastatic gestational choriocarcinoma. The patients' clinical history, imaging, and laboratory results were retrieved from the electronic medical records. The initial pathology diagnostic work-up consisted of histo-morphologic examination and immunohistochemical stains (initial immunohistochemical panel varied among the three cases), including panCK, CK AE1/AE3, CK7, CK5-6, EMA, CD45, S100, TTF-1, napsin A, p40, SALL4, beta-hCG, hPL, GATA3, PAX8, ER, and CDX2. Short tandem repeat genotyping was performed in all cases to confirm or to rule out gestational origin of the tumor.

For short tandem repeat genotyping a formalin-fixed paraffin-embedded block containing tumor and normal tissue was selected and 10 unstained sections were created. Tumor and normal tissues were scraped separately from the unstained sections using a sterile scalpel into a microcentrifuge tube. DNA was extracted by hydrothermal pressure method of simultaneous deparaffinization and lysis of formalin-fixed paraffin-embedded tissue followed by conventional column purification to obtain high quality DNA [17]. Tissue genotyping using PowerPlex® 16 System (Promega Corporation, Madison, WI, USA) was performed by multiplex polymerase chain reaction (PCR) at 15 short tandem repeat loci according to manufacturer instruction. One microliter of the PCR product was mixed with 13 µL of Hi-Di and 0.5 µL sizing marker (GeneScan-500LIZ, Applied Biosystems, Inc.), followed by capillary electrophoresis on an ABI3130 platform. Data collection and analysis were performed using GeneMapper software version 3.7 (Applied Biosystems, Inc., Foster City, California, USA). PCR products were identified by fluorescent color and expected size range. The genotype of normal tissue was compared with the tumor genotype at each locus to assess if the tumor contains unique paternal alleles (from a prior or concurrent gestation) that are not present in the patient's normal tissue.

Results

Case presentations

The clinical presentations and follow-up information of all patients are summarized in Table 1.

Case #1

A 37-year-old gravida 4 para 3103 patient presented in the emergency room with a sudden onset of chest pain, shortness of breath, hemoptysis, and abdominal pain. CT scan revealed multiple lung nodules bilaterally up to 2.8 cm in size (Fig. 1a) and a 7.6 cm hemorrhagic mass in the right pelvic sidewall. CT and MRI of the brain were unremarkable. Her clinical history was significant for delivery of a stillborn fetus at 35 weeks 3 days gestational age, complicated by postpartum hemorrhage requiring massive transfusion and supracervical hysterectomy 2 months prior to her presentation. Her initial serum beta-hCG was 520,000 mIU/mL and a core needle biopsy of the left lung was performed. The biopsy revealed clusters of markedly atypical tumor cells with extensive necrosis and hemorrhage. Scattered necrotic multinucleated tumor cells were also present. Immunostains for panCK and beta-hCG were positive in tumor cells, while CD45, S100, TTF-1, hPL, p40, and SALL4 were negative (Fig. 1b–d). Based on the overall clinical impression, morphology and immunoprofile the initial diagnosis was reported as metastatic carcinoma, consistent with choriocarcinoma.

Short tandem repeat genotyping was ordered for further evaluation of possible gestational origin and showed unique alleles in the tumor tissue at 9 of the 15 analyzed loci, that were not present in the patient's normal tissue, consistent with paternal alleles from a prior biparental gestation (Fig. 2), consistent with gestational choriocarcinoma.

Retrospective microscopic examination of the placenta from the patient's most recent pregnancy and the supracervical hysterectomy specimen revealed no evidence of choriocarcinoma or any other significant abnormality. Genotyping of the placenta showed identical genetic profiles with the choriocarcinoma, confirming the causal link between the two tissues (Fig. 2).

The tumor was staged as pT1 M1a, Stage III [18], with a FIGO risk score of 8 [2], and the patient received one cycle of cisplatin-etoposide, followed by 13 cycles of EMA-CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine). Her serum beta-hCG decreased gradually and was <1 mIU/mL after the 10th chemotherapy cycle (5 months after the first cycle). After completion of chemotherapy follow-up imaging showed a persistent right adnexal mass measuring 6 × 6 cm, and the patient underwent right salpingo-oophorectomy. Microscopic

Table 1 Clinical presentation and follow-up

Case #	Patient age (year)	History of prior gestations	Preoperative Serum beta-hCG	Pelvic imaging	Lung imaging	Preliminary diagnosis	Molecular genotyping	Final diagnosis	Treatment	Follow-up
1	37	G4P3103, last pregnancy 2 months prior, IUFD at 35 weeks	520,000 mIU/mL	7.6 cm right pelvic sidewall mass	Multiple nodules bilaterally (up to 2.8 cm)	Metastatic carcinoma, consistent with choriocarcinoma	Distinct paternal alleles present; balanced biparental gestation	Metastatic gestational choriocarcinoma	1 cycle EP, 13 cycles EMA-CO	17 months; no evidence of disease
2	41	G5P2032, last pregnancy 6 years prior (miscarriage at 5 months)	10,727 mIU/mL	Endometrial thickening	9.7 cm mass in RUL and 5 mm mass in LLL. Right hilar lymphadenopathy.	Carcinoma with trophoblastic differentiation	Identical genetic profiles between tumor and normal tissue	Primary lung carcinoma with trophoblastic differentiation	3 cycles of MTX for suspected ectopic pregnancy. After final pathology diagnosis 6 cycles of carboplatin paclitaxel, followed by pembrolizumab	15 months; dead of disease
3	48	G3P2, last pregnancy 6 years prior (NSVD)	Not evaluated	Normal	1.5 cm nodular structure in RLL with right hemothorax	Large cell carcinoma	Distinct paternal alleles present; balanced biparental gestation	Metastatic gestational choriocarcinoma	EMA-CO	4 months; no evidence of disease

IUFD intrauterine fetal demise, *RUL* right upper lobe, *LLL* left lower lobe, *EMA-CO* etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine, *MTX* Methotrexate, *NSVD* normal spontaneous vaginal delivery

examination showed extensive necrosis and hemorrhage without viable tumor tissue. The patient is alive and well with no evidence of disease at 17 months follow-up and her serum beta-hCG level continues to be <1 mIU/mL.

Case #2

A 41-year-old gravida 5 para 2032 patient presented with irregular bleeding for 4 months and positive urine pregnancy test. The patient's last prior pregnancy was 6 years ago and transvaginal pelvic ultrasound revealed thickened endometrium at 16.5 mm and no evidence of intrauterine pregnancy or a pelvic mass lesion. An endometrial curettage was performed and showed inactive endometrium with stromal pseudo-decidual change. No trophoblast, chorionic villi, or fetal parts were identified. Based on these findings the clinical diagnosis of ectopic pregnancy was made and the patient received three cycles of methotrexate. The initial serum beta-hCG level was 10,727 mIU/mL which failed to decrease despite chemotherapy. CT scan of the chest revealed a 9.7 × 6.7 cm lung mass in the right upper lobe with right hilar lymphadenopathy (Fig. 3a) and a 5 mm nodule in the left lower lobe. No additional abnormalities were identified on imaging studies of the abdomen, pelvis, and brain at that time.

Core needle biopsy of the right lung mass showed solid nests of poorly differentiated carcinoma with marked nuclear pleomorphism, focal necrosis and hemorrhage, and scattered multinucleated tumor cells. The tumor cells were diffusely positive for CK AE1/AE3, beta-hCG, and GATA3 immunostains and showed focal immunoreactivity with EMA, and p40 (Fig. 3b–d). TTF-1 and CK5-6 immunostains were negative. PD-L1 immunostain showed 30% of tumor cell staining and 15% of immune cell staining within the tumor. The preliminary diagnosis of carcinoma with trophoblastic differentiation was rendered and short tandem repeat genotyping was ordered to assess the possibility of gestational origin.

Short tandem repeat genotyping found loss of heterozygosity within the tumor at three loci, and no distinct paternal alleles within the tumor compared with the patient's normal tissues to suggest a gestational origin (Fig. 4). Based on the morphology, immunophenotype, and genotyping results the final diagnosis was consistent with primary lung carcinoma with trophoblastic differentiation.

Targeted next generation sequencing using the 146-gene Oncomine™ assay identified mutations in *SMARCA4, ARID1A, NF2, ATR, CDK12*, and *POLE*. No gene amplifications or fusions were detected. Fluorescent in situ hybridization (FISH) did not identify rearrangements in *ALK* or *ROS1* genes.

The patient was started on carboplatin paclitaxel chemotherapy and received a total of six cycles.

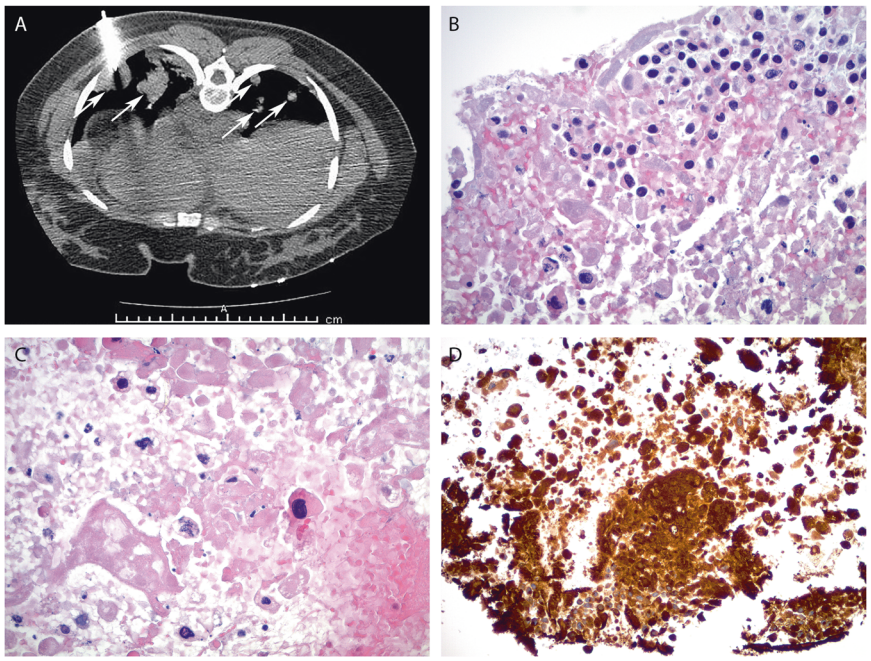

Fig. 1 Case #1. CT of the chest identified multiple lung nodules bilaterally (arrows) up to 2.8 cm in size (**a**). Core needle biopsy of one of the lung nodules revealed clusters of tumor cells with marked atypia and extensive necrosis (**b**). Scattered necrotic tumor cells appeared to show a multinucleated, syncytiotrophoblastic morphology (**c**). Beta-hCG immunostain was strongly and diffusely positive (**d**)

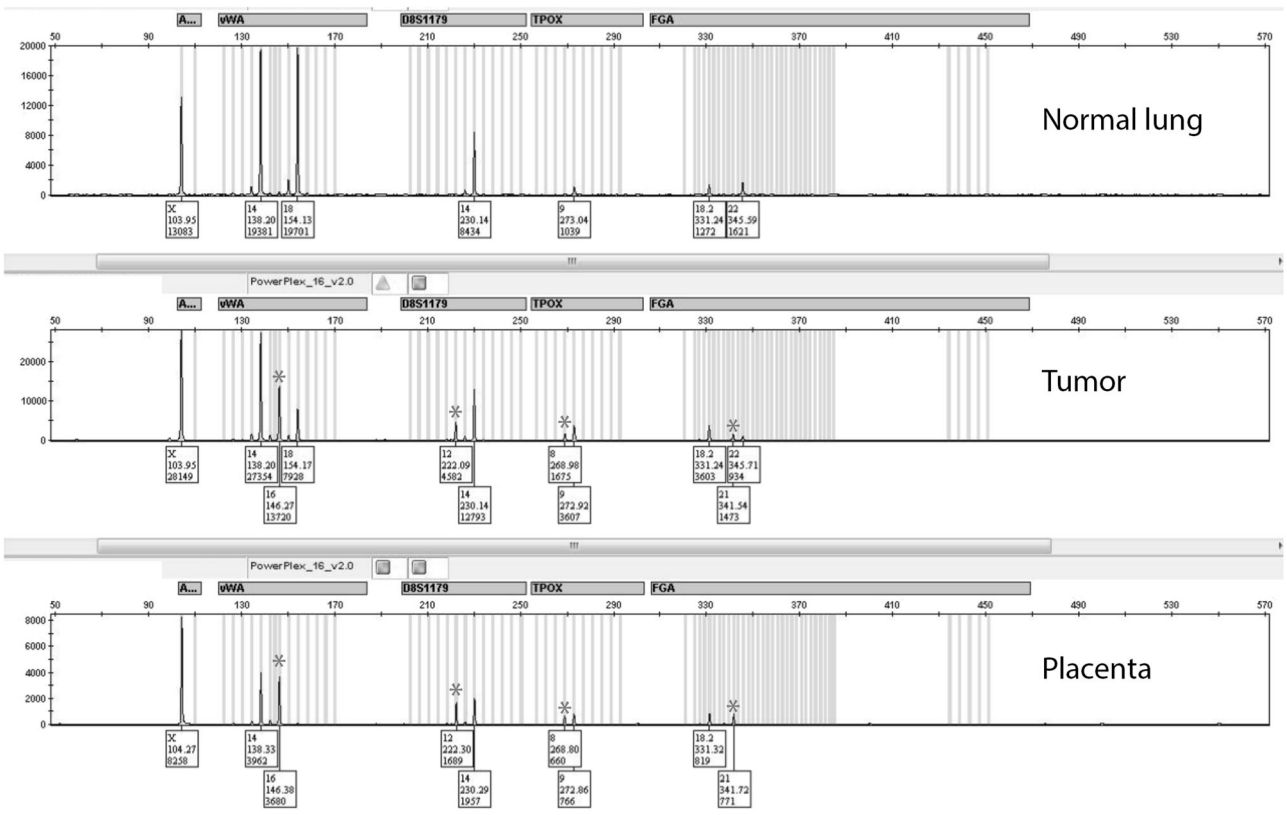

Fig. 2 Case #1. Genotyping analysis of the lung tumor showed presence of unique paternal alleles (red asterisk) matching with the placenta from the patient's antecedent pregnancy, confirming the diagnosis of metastatic gestational choriocarcinoma arising from the patient's most recent near-term pregnancy

At 3 months follow-up PET-CT revealed enlargement of the dominant pulmonary mass to 11.8 cm, along with multiple new lung nodules, a new left hepatic lobe mass, and a hypermetabolic focus in the left iliac bone. Eight months after her initial presentation she developed a brain metastasis and was treated with gamma

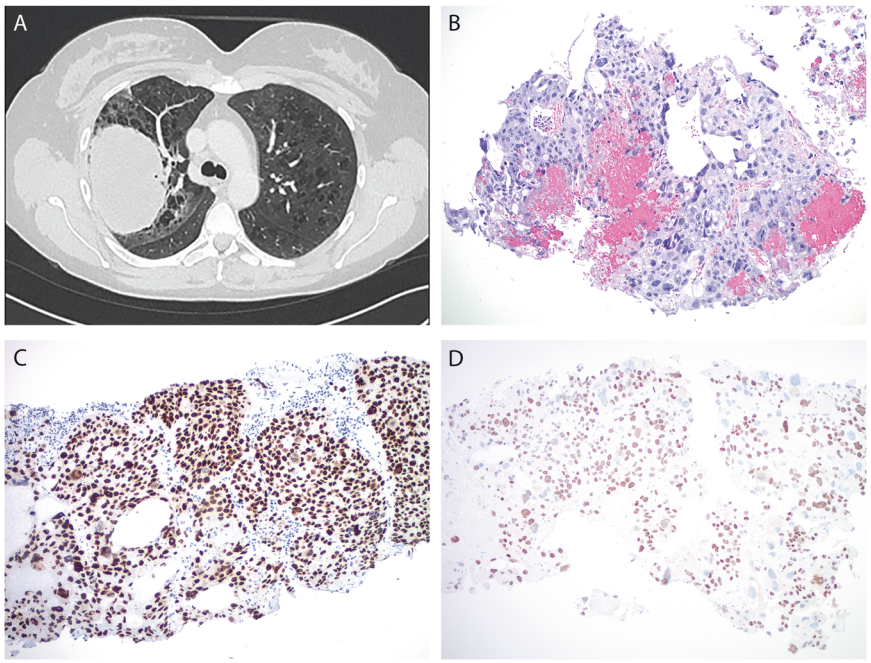

Fig. 3 Case #2. CT of the chest identified a large solitary lung mass in the right upper lobe with right hilar lymphadenopathy and emphysema (**a**). Core needle biopsy of the lung mass showed solid sheets of tumor cells with marked nuclear pleomorphism, multi-nucleation, and focal hemorrhage and necrosis (**b**). GATA3 immunostain was strongly and diffusely positive (**c**) and p40 immunoreactivity was present in the majority of tumor cells (**d**)

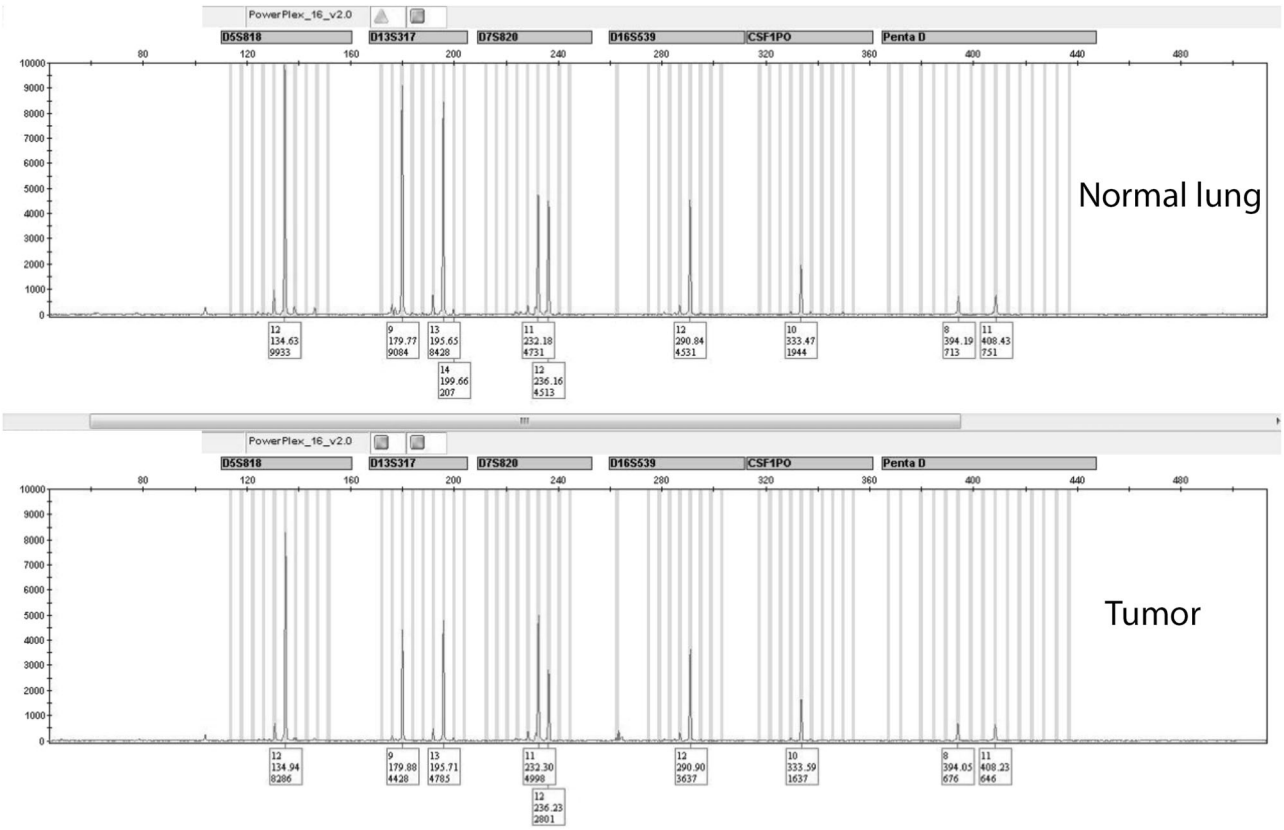

Fig. 4 Case #2. Genotyping analysis showed matching allelic profiles between the patient's normal lung tissue and the tumor, with no unique paternal alleles present, consistent with a primary lung carcinoma. Loss of heterozygosity was observed within the tumor at three loci (not shown)

knife resection. Due to systemic progression of disease, the patient was started on pembrolizumab; however, the disease continued to progress requiring right parietal craniotomy with resection of tumor and the patient died of the disease 15 months after her initial presentation.

Case #3

A 48-year-old gravida 3 para 2 woman presented to the emergency room with right pleuritic chest pain and chest CT revealed a large right-sided hemothorax and a 1.5 cm nodular structure in the right lung. The patient's last pregnancy 6 years prior was a term spontaneous delivery complicated by retained placenta. An emergency thoracoscopy and right lower lobe wedge resection was performed along with evacuation of ~1 l of hemothorax. CT of the head, MRI of the brain, and CT of abdomen and pelvis showed no abnormalities. Serum beta-hCG measurement was not considered preoperatively.

Gross examination of the specimen showed a 12 × 5.5 × 2.5 cm lung wedge with a 7 × 5 cm area of pleural disruption. Sectioning revealed a 7 × 3 × 2.5 cm centrally cystic, hemorrhagic, and friable mass lesion associated with the pleural defect (Fig. 5a). Microscopically over 90% of the mass was necrotic, and the viable tumor tissue at the periphery formed large cohesive, solid sheets composed of markedly pleomorphic tumor cells with scattered multinucleated giant cells (Fig. 5b–d). Vascular invasion was present. Immunohistochemical stains for CK7 and GATA3 were diffusely and strongly positive, PAX8 showed diffuse weak to moderate immunoreactivity, while p40, TTF-1, Napsin A, CK5-6, OCT4, ER, and CDX2 were negative in tumor cells. Based on the microscopic evaluation and immunohistochemical results the initial pathology diagnosis of large cell carcinoma was made. Consultation with a gynecologic pathologist raised the possibility of choriocarcinoma and short tandem repeat genotyping was pursued.

Genotyping found unique alleles in the tumor tissue at 11 of the 15 analyzed loci, consistent with paternal alleles from a prior biparental gestation, confirming metastatic gestational choriocarcinoma (Fig. 6).

Targeted next generation sequencing of 50 genes by the Ion Ampliseq™ Cancer Hotspot Panel v2 did not identify any mutations. Fluorescent in situ hybridization (FISH) did not identify rearrangements in *ALK* or *ROS1* genes.

A completion right lower lobectomy was performed with lymph node sampling and showed no residual carcinoma. Postoperative PET scan showed non-specific postsurgical changes without evidence of metastatic disease. The immediate postsurgical serum beta-hCG level was 315 mIU/mL, which showed a temporary decline to 185 mIU/mL and then continued to rise again to 671 mIU/mL 2 weeks after her surgery.

The tumor was staged as pT1 M1a, Stage III [18] with a FIGO risk score of 8 [2] and the patient was started on EMA-CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine) and her serum beta-hCG normalized after two cycles of chemotherapy. She is doing well without evidence of disease 4 months after her initial presentation.

Discussion

Tumors with trophoblastic differentiation present a unique clinical and pathological diagnostic challenge in female patients. The pathogenesis—gestational, germ cell, or somatic origin—essentially defines the specific entity and determines the patient's prognosis and best therapeutic

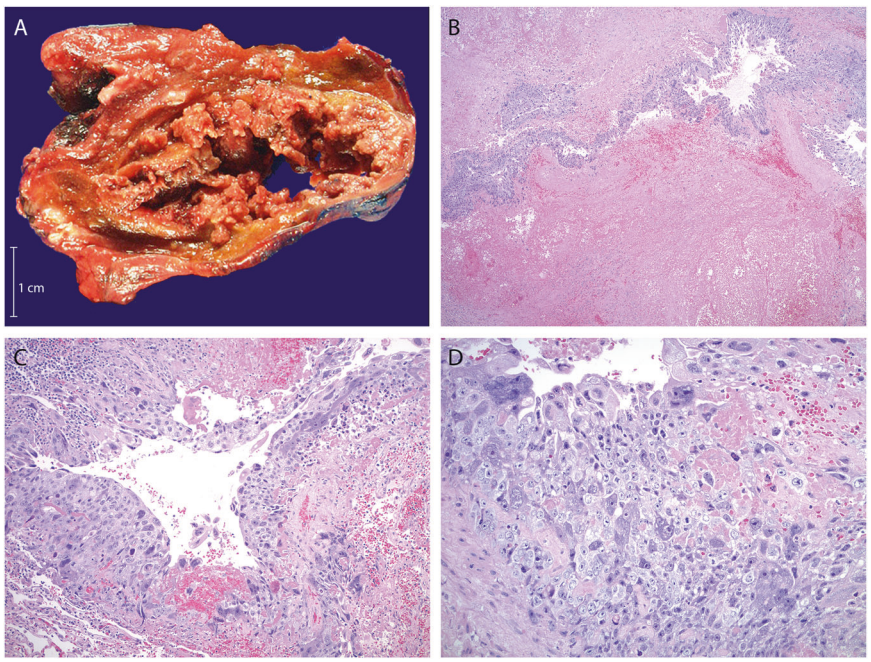

Fig. 5 Case #3. Gross examination of the right lower lobe wedge resection specimen revealed a 7 cm cystic, hemorrhagic, and friable mass lesion (**a**). Microscopic sections showed extensive necrosis and hemorrhage with rare foci of viable tumor at the periphery (**b**). The tumor cells had a biphasic arrangement with sheets of large mononuclear cells rimmed by multinucleated giant cells (**c**, **d**)

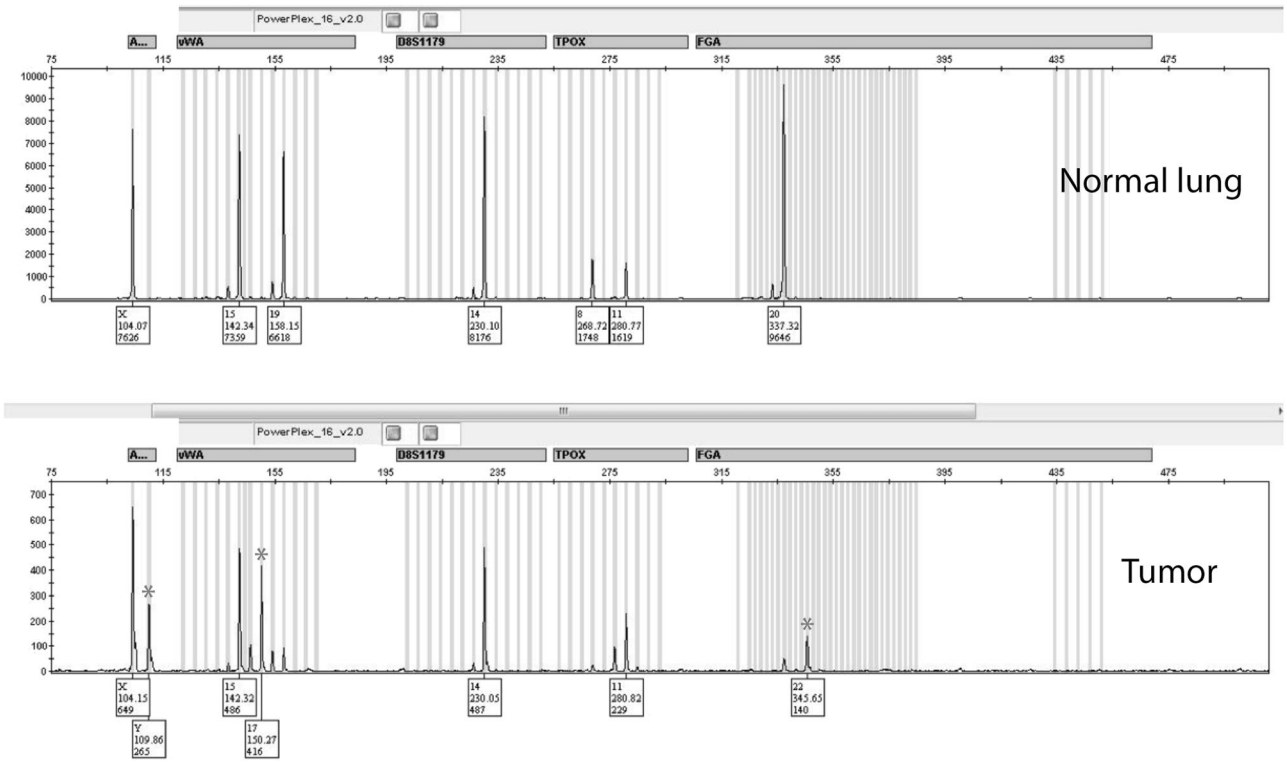

Fig. 6 Case #3. Genotyping analysis identified unique paternal alleles within the tumor (red asterisk), including one on the Y chromosome (first locus on the left side of the image), consistent with metastatic gestational choriocarcinoma

strategy. The clinical presentation—patient age, menopausal status, detailed history of prior gestational events, including intrauterine or ectopic pregnancies, abortions, complete or partial hydatidiform mole, imaging studies (sites of involvement), and laboratory work-up (serum beta-hCG levels)—often helps narrow the differential diagnosis. However, some cases may show significant overlap in their clinicopathological features and require additional diagnostic modalities.

All three lung tumors in our series presented in premenopausal young women without definitive clinical evidence of gestational origin. Serum beta-hCG level was evaluated only in two of the cases, both of which were over 10,000 mIU/mL, and the clinical impression favored gestational choriocarcinoma in both of them. Microscopic examination and genotyping analysis confirmed gestational choriocarcinoma in the first case; however, gestational origin was ruled out in the second case. Gestational trophoblastic disease was not suspected clinically in the third patient, who presented with acute shortness of breath, hemothorax, and a solitary lung nodule. Based on the initial microscopic assessment primary large cell lung carcinoma was favored, yet genotyping proved gestational origin of the tumor.

Gestational choriocarcinoma typically presents during the reproductive years (average age of 30 years), following a normal pregnancy, complete hydatidiform mole or abortion in 50%, 22.5%, and 20% of the cases, respectively [2, 4]. The risk of choriocarcinoma after a complete mole is 2–3%, while it is extremely rare following a partial hydatidiform mole (<0.5%) [19]. The most common clinical symptom is vaginal bleeding, but the first presentation may also include extrauterine hemorrhage as a result of lung, brain, liver, kidney, and gastrointestinal tract metastases [3]. The time interval between choriocarcinoma and the antecedent gestation is usually a few months: 1–3 months on average after a term pregnancy and 13 months following a complete mole, although rarely it may be over 20 years in some cases [20, 21]. The serum beta-hCG level usually exceeds 10,000 mIU/mL and may even be over 1,000,000 mIU/mL [22]. The tumor typically forms a bulky, extensively hemorrhagic and necrotic mass lesion within the endo-myometrium, but it may also arise from the uterine cervix, and from the fallopian tube or ovary in association with an ectopic pregnancy [23]. In rare cases the primary tumor may be intraplacental requiring careful gross and microscopic examination of the term placenta [24, 25]. In contrast, non-gestational choriocarcinoma of germ cell origin in female patients is very rare, usually occurs in children or in young adults, involves the ovary and may contain other non-choriocarcinomatous components as part of a mixed germ cell tumor [26]. Somatic carcinomas at

various primary sites can also show trophoblastic differentiation—in the form of a choriocarcinomatous component or as scattered syncytiotrophoblastic giant cells, mimicking metastatic gestational choriocarcinoma. Helpful clinical features in favor of somatic origin include older patient age, postmenopausal status, and a relatively lower level of serum beta-hCG (usually <10,000 mIU/mL) [8, 11].

Microcopically choriocarcinoma, regardless of its histogenetic origin, shows a bi- or triphasic growth pattern composed of mononuclear trophoblastic cells and multinucleated syncytiotrophoblasts. The nuclear atypia is marked, often with bizarre nuclei and there is brisk mitotic activity with frequent atypical mitotic figures. Abundant tumor necrosis and hemorrhage are present. In addition, somatic carcinomas with trophoblastic differentiation typically also contain a recognizable somatic carcinoma component, e.g., adenocarcinoma, clear cell carcinoma, squamous cell carcinoma, giant cell carcinoma, or urothelial carcinoma [8, 11, 12, 14, 27]. However, the somatic carcinoma component in these cases may only be focal requiring thorough sampling, and may not always be present in a small biopsy specimen.

Immunohistochemical stains do not allow for pathogenetic distinction, but can be helpful confirming trophoblastic differentiation: GATA3 and inhibin are not specific, but can be used as pan-trophoblastic markers, hCG usually shows diffuse immunoreactivity and hPL expression is variable [28, 29]. Interestingly, SALL4 immunostain—a frequently used germ cell marker—has been recently reported to be positive in gestational choriocarcinomas, thus SALL4 expression should not be used to distinguish between choriocarcinoma of germ cell vs. gestational origin [30, 31]. SALL4 expression has also been described in poorly differentiated somatic carcinomas at various primary sites, with the highest proportion of SALL4 positive tumors observed in the ovary, stomach, and pancreas [30]. Among lung tumors SALL4 expression is most commonly seen in small cell carcinomas (19%), but 0.8–6% of lung adenocarcinomas are also SALL4 positive [30, 32]. One of the gestational choriocarcinomas in our series (case #3) also showed diffuse PAX8 reactivity. PAX8 expression has been previously observed in term placenta in one study [33]; however, the literature on PAX8 in gestational trophoblastic tumors is limited. A tissue microarray study of PAX8 expression in human epithelial tumors failed to show PAX8 staining in any of the four gestational neoplasms included in their cohort [34].

Molecular analysis of tumor tissue to identify unique paternal genetic contribution from a prior gestational event provides a definitive diagnostic distinction between tumors of gestational and non-gestational origin. Fisher et al. used restriction fragment length polymorphism (RFLP) analysis to classify the histogenesis of choriocarcinomas in three patients [35]. More recent papers reported microsatellite analysis of tumor and the patient's normal tissue as well their partner's DNA to confirm gestational origin of trophoblastic tumors and to precisely identify the causative gestational event [5, 7, 21, 36, 37]. Short tandem repeats or microsatellites are highly prevalent, noncoding, repetitive DNA sequences of 2–7 nucleotides in the human genome and are genetically stable. The number of repeats differs between individuals and thus, the genetic profiles of individuals can be distinguished from each other by identification of the number of short tandem repeats at specific loci [38, 39]. By the same principle, short tandem repeat genotyping and comparison of genetic profiles between maternal and chorionic villous tissue offers a determination of parental genomic contribution and therefore can diagnose and subclassify hydatidiform moles at the genetic level [40, 41]. Genotyping is now an integral part of the diagnostic algorithm for molar gestations at many large academic centers [42, 43]. In tumors with trophoblastic differentiation, comparison of the short tandem repeat loci between tumor tissue and the patient's normal tissue provides information about the presence or absence of unique paternal alleles in the tumor allowing for determination of gestational or non-gestational origin, respectively. However, genotyping analysis is unable to distinguish between a choriocarcinoma of germ cell origin and a somatic carcinoma with trophoblastic differentiation, due to lack of unique paternal alleles in both entities. In gestational trophoblastic tumors, genotyping also allows for precise identification of the causative gestational event (hydatidiform mole, abortion, or term pregnancy). The type and time interval since the causative gestation, which may not be the patient's immediate antecedent pregnancy, plays a significant role in the clinical risk assessment of gestational trophoblastic disease and therefore determines the treatment protocol (single agent vs. combination chemotherapy) [2].

Short tandem repeat genotyping can also potentially identify loss of heterozygosity (LOH) within the tumor, as was seen in case #2 in our series. However, LOH is a common finding across all cancer types and is not specific to a tissue of origin or a certain pathogenetic pathway. Nichols et al. have recently identified LOH in 16% of genes on average in over 9000 patients in 33 different tumor types in The Cancer Genome Atlas database [44].

Our study highlights the complex clinicopathological diagnostic challenges posed by trophoblastic differentiation encountered in lung tumors in young women. The clinical presentation may not be typical for a gestational choriocarcinoma, as the tumor may be solitary and may develop years after the patient's last pregnancy without abnormal uterine bleeding or an overt pelvic mass lesion as was seen in one of our cases (case #3). In another case (case #2) the clinical presentation—positive urine pregnancy test,

elevated serum beta-hCG, and endometrial thickening—was concerning for a gestational choriocarcinoma, yet the tumor genotype was consistent with a primary pulmonary carcinoma. Gestational choriocarcinoma is highly chemosensitive and responds well to single agent methotrexate (low risk disease) or EMA-CO combination chemotherapy (high risk disease) with an excellent prognosis [2]. Primary lung carcinomas with trophoblastic differentiation, on the other hand, typically have an aggressive clinical course with poor response to chemotherapy [10]. In our series, both patients with gestational choriocarcinoma received EMA-CO regimen and achieved complete response with normalization of serum beta-hCG levels and no evidence of disease at their last follow-up. However, the patient with primary lung carcinoma experienced rapid disease progression with liver, bone and brain metastases despite combination chemotherapy and died 15 months after her initial presentation.

In conclusion, presentation of a high-grade carcinoma in the lung of a young woman poses a unique diagnostic challenge in the absence of history of gestational trophoblastic disease or a pelvic mass. High index of suspicion is crucial and the differential diagnosis of metastatic gestational trophoblastic tumor must be considered before triaging small tumor biopsy specimens for reflex molecular testing as primary lung carcinoma. Molecular genotyping is a powerful tool for separation of primary lung carcinoma from a metastatic trophoblastic tumor of gestational origin with profound therapeutic and prognostic implications for the patient.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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