Pathology and Genomics in Gestational Trophoblastic Neoplasia

13

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Abstract

Gestational trophoblastic diseases (GTDs) originate from placental tissue and are rare tumors with a current cure rate of greater than 90% with the right diagnosis and clinical management. GTDs are generally divided into two categories: (1) hydatidiform moles, presenting abnormal villous proliferation with chromosomal aberrations, and (2) rare gestational trophoblastic neoplasms (GTNs) including choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor. Persistent gestational trophoblastic disease/tumor most commonly occurs following molar pregnancy; however, it may follow any GTD.

Hydatidiform mole was previously diagnosed in the second trimester; however, it is now diagnosed in first trimester specimens, based on the availability of accurate and sensitive tests for the detection of hCG and on the use of early ultrasonographic examination. Often, a diagnosis is made before classical clinical signs and symptom develop. In daily practice, histological diagnosis of GTD continues to have some degree of diagnostic misclassification with a fairly high degree of inter- and intraobserver variability. Studies evaluating the concomitant use of histology with $p57^{KIP2}$ immunohistochemistry, and/or genotyping, have further refined diagnoses of hydatidiform mole. Beyond hydatidiform mole, the even rarer tumors of the GTN family require broad knowledge of the clinical and histological features, as well as the application of immunohistochemical markers directed toward the various types of trophoblast, to arrive at the correct diagnosis.

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This chapter focuses on recent advances in the pathogenesis; pathological diagnostic features, including immunohistochemistry; and genetic findings, of GTD, along with a review of the clinical management.

Keywords
Hydatidiform mole • Choriocarcinoma • PSTT • ETT

13.1 Molar Pregnancies

WHO classification (the 4th edition, 2014) of gestational trophoblastic disease (GTD) includes neoplasms, molar pregnancies, nonneoplastic lesions, and abnormal (non-molar) villous lesions [1] (Table 13.1). Molar pregnancies include complete hydatidiform mole (CM), partial hydatidiform mole (PM), and invasive mole. Hydatidiform mole is an abnormal placenta with villous hydrops and variable degrees of abnormal trophoblastic hyperplasia which can be distinguished by means of gross morphologic and histopathological examination along with cytogenetic analyses.

The prevalence of hydatidiform mole varies by country, with the highest incidence in Southeast Asia (3.8–13/1000 pregnancies) and the lowest incidence in the USA and Europe (0.5–1.84/1000 pregnancies) [1–3]. In Japan, the incidence is decreasing from 2.5/1000 pregnancies in 1974 to 1.65/1000 pregnancies in 2000 [4].

Significant risk factors are maternal age >40 years, previous spontaneous abortion and previous history of hydatidiform mole, and Asian ethnicity and genetics. Some studies suggest that vitamin A deficiency and nutritional and socioeconomic factors may increase the risk of molar pregnancy [1, 3, 5].

Persistent GTD is determined by hCG values that have plateaued or are increasing after curettage for hydatidiform mole. Further clinical and imaging studies are indicated to exclude invasive mole or high-risk GTD including choriocarcinoma [5–7]. The incidences of persistent GTD are 15–29% following CM and 0–4% after PM [1, 6]. It is crucial to diagnostically separate the non-molar, hydropic abortions from hydatidiform moles for prognostic and clinical management purposes.

Table 13.1 Classification of gestational trophoblastic disease (WHO classification, 2014)

Neoplasms	Choriocarcinoma
	Placental site trophoblastic tumour
	Epithelioid trophoblastic tumour
Molar pregnancies	Hydatidiform mole
	• Complete
	• Partial
	• Invasive
Non-neoplastic lesions	Exaggerated placental site
	Placental site nodule and plaque
Abnormal (nonmolar) villous lesions	

The clinical presentation of a CM has changed considerably over the past three decades. CM was once easily diagnosed in the second trimester, and certain symptoms were common at the time of presentation, including prominent uterine enlargement, anemia, toxemia, hyperemesis, hyperthyroidism, and respiratory failure. However, the diagnosis is now typically made in the first trimester often before classic clinical symptoms develop. This is based on the availability of accurate and sensitive tests for hCG and the widespread use of both transabdominal and transvaginal ultrasonographies [5, 6].

Although abortion specimens with hydropic chorionic villi are routinely encountered in general and gynecological pathology practice, the histological features of early CM (at less than 12-week gestational age), PM, and hydropic abortus often overlap and have a low sensitivity and specificity, especially for PM [8].

The development of ancillary diagnostic testing methods, including immunohistochemical detection of imprinted genes/products, DNA ploidy analysis, and, most recently, DNA short tandem repeat (STR) genotyping, has advanced the study of GTD during the past three decades [9–11]. Diagnostic algorisms have been proposed in pathological diagnosis of PTD with the concomitant use of traditional histopathological assessment and ancillary studies for higher diagnostic accuracy [9, 10].

Clinical and pathological diagnostic features of hydatidiform moles and nonmolar hydropic abortus are summarized in Table 13.2.

13.1.1 Compete Hydatidiform Mole

WHO classification (2014) defines CM as a nonneoplastic, proliferative disorder of the placenta, resulting in villous hydrops and trophoblastic hyperplasia without embryonic development and having androgenic diploid karyotype (diploid paternal-only genome) [1].

Clinical findings are vaginal bleeding in the second trimester, prominent uterine enlargement, and marked elevation of serum hCG (>100 × 10^3 mIU/mL). Ultrasonography shows the absence of fetus and "snow storm" pattern. These are characteristic features of well-developed, classic CM [6].

Genetically, the majority of CM cases have a diploid, paternal-only genome with the karyotypes of 46XX or 46XY. Two paternal haploid chromosome sets consist of either monosomic/homozygous (80–90%) or dispermic/heterozygous (10–20%) origin (Fig. 13.1) [1, 3]. Rarely, tetraploid CM may exist with four paternal haploid sets in the genome.

Macroscopically, classic CM consists of bulky, bloody tissue with uniformly hydropic "grapelike" villi (Fig. 13.2) [1, 5, 6]. The edematous villi range from a few millimeters to over 10 mm in diameter. Fetal and normal placenta are absent apart from rare exceptions [12, 13]. Early complete mole (ECM) of 6.5 to 12 weeks of gestational age is typically normal grossly [6].

Histological features of classic CM differ from those of ECM. Knowledge of the gestational age is useful in determining the appropriate histological criteria to be

Table 13.2 Diagnostic features of hydatidiform moles

Features	CM	ECM (6.5–12 weeks of gestation)	PM	Hydropic abortus
Karyotype	46XX, 46XY (paternal-only)	46XX, 46XY (paternal-only)	69XXX, 69XXY, 69XYY	46XX, 46XY
Pretreatment hCG (mIU/mL)	$>100 \times 10^3$	Normal or $<100 \times 10^3$	Normal or $<100 \times 10^3$	Normal
Ultrasound	Snow storm pattern	–	Focal cystic change	–
Gestational sac and fetus	Absence	Absence	Rarely presence	Presence
Macroscopy	Overall hydropic change	No gross abnormality	Focal hydropic change	No gross abnormality
Villous shape				
Outline	Round to oval	Polypoid Cauliflower shapes Crab-shaped	Scalloped with pseudo-inclusionfjord-like invagination	Round to oval
Enlargement	Marked	Normal size	Some enlarged, but often not prominent	Often marked
Cistern	Prominent	Uncommon	Variable, usually not prominent	Variable, usually not prominent
Trophoblastic hyperplasia				
Extent	Multifocal	Circumferencial	Focal, syncytiotrophoblast knuckles (sprouts)	None
Amount	Abundant	Increase	Minimal	None
Atypia	Marked	Mild to moderate	Limited to mild	None
Normal villi	None or few	Some	Numerous	Sometimes
Apoptosis in villous stroma	Marked	Marked to mild	Rare	None
Vasculature and nucleated RBCs	Absent, generally	Absent, capillary may be present	Common	Absent, generally
p57KIP2 immunostain in cytotrophoblast and villous stromal cells	Negative	Negative	Positive	Positive
Persistent trophoblastic disease	15–29%	15–29%	0–4%	0%

CM complete hydatidiform mole, *ECM* early complete hydatidiform mole, *PM* partial hydatidiform mole, *HA* hydropic abortus, *hCG* human chorionic gonadotropin

applied. Classic CM presents with diffuse villous enlargement and edema, frequent cistern formation, and conspicuous trophoblastic hyperplasia. Cistern formation is cavitation in the center of the villi produced by necrosis of the mesenchyme (Fig. 13.3a). Villous stromal vessels are usually absent. The villi are usually round to

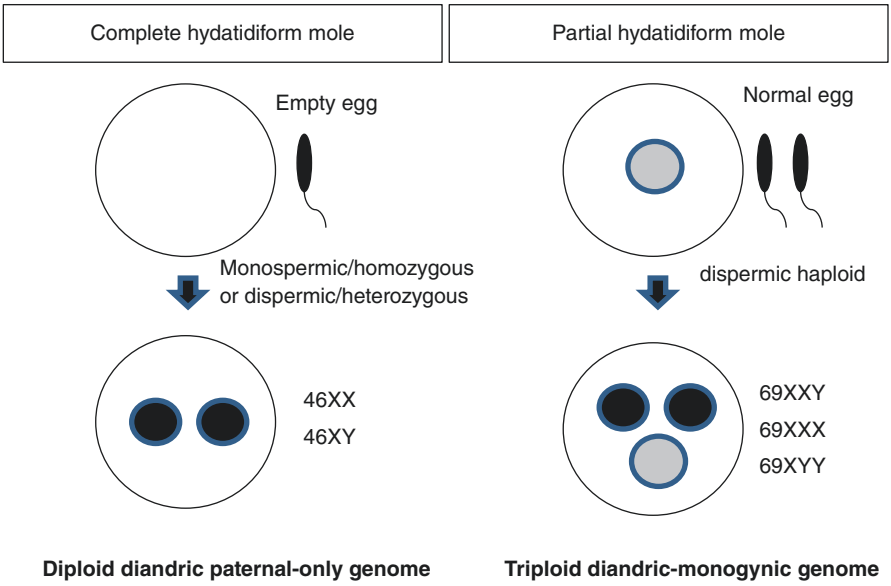

Fig. 13.1 Pathogenesis of complete hydatidiform mole and partial hydatidiform mole

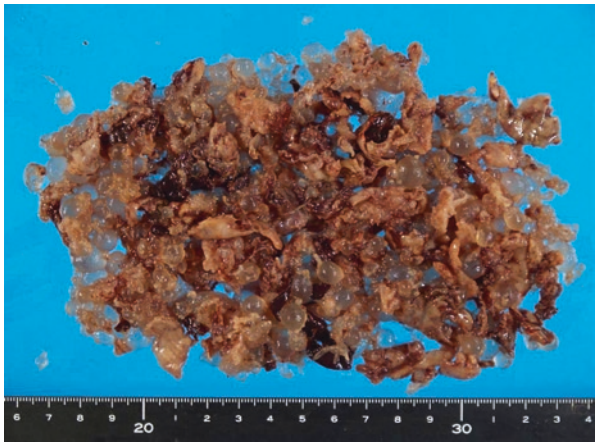

Fig. 13.2 Gross appearance of complete hydatidiform mole presenting diffuse grapelike villous swelling

oval. Trophoblastic hyperplasia is circumferential, and significant cytological atypia of all three trophoblasts is almost always present (Fig. 13.3b). Mitotic figures are usually found. There are no fetal tissue and normal placental structures excluding very rare exceptions. In contrast, ECM shows minimal hydropic change and cistern formation is rare [14, 15]. The villi have irregular shapes called polypoid, cauliflower-like, or crab-shaped (Fig. 13.3c). Trophoblastic hyperplasia is mild to moderate in degree, and trophoblast shows circumferential or random distribution on the villi. An exaggerated placental site (molar implantation site) with atypical trophoblast is often present. The villous stroma is abnormally cellular with prominent apoptosis (karyorrhexis)

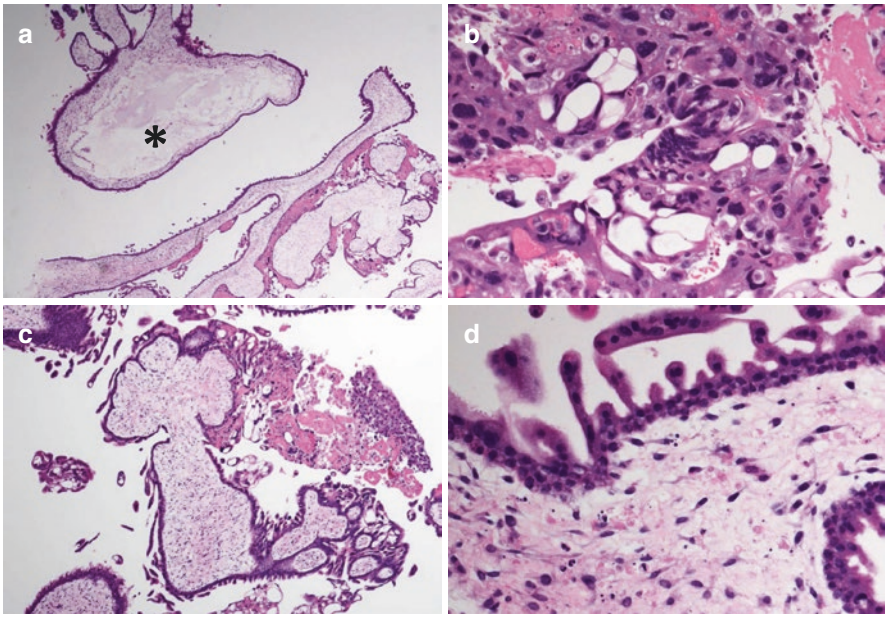

Fig. 13.3 Histological appearance of complete hydatidiform mole. (**a**) Swollen villi with necrosis of the mesenchyme produce cistern (*), and left lower side villi show polypoid, crab-shaped irregular outline. (**b**) The implantation site trophoblast presenting conspicuous trophoblastic atypia. (**c**) Early complete hydatidiform mole. Characteristic irregular villous contour resembling cauliflower-like shape. (**d**) Early complete hydatidiform mole. Hypercellular myxoid villous stroma with karyorrhexis (apoptosis) accompanied by modest circumferential trophoblastic proliferation

and myxoid change (Fig. 13.3d) [14, 15]. Capillary vessels can be seen in the stroma with or without nucleated red blood cells. Stromal fibrosis is absent.

The prevalence of persistent gestational trophoblastic disease is 15–29%, and 2–3% of the patients develop choriocarcinoma after CM [1, 3, 6]. The risk of subsequent CM is 1–1.8% and 10–18% after two consecutive CMs [16, 17]. The rare case of recurrent, familial, and biparental CM develops as a result of abnormal imprinting and overexpression of the paternal genome related to maternal mutation of *NALP7/NLRP7* and more rarely *KHDC3L* [3, 18].

13.1.2 Partial Hydatidiform Mole (PM) (Figs. 13.5, 13.6 and 13.7a–d)

PM is defined as a hydatidiform mole with a spectrum of villous populations ranging from normal size to substantial hydrops with mild, focal trophoblastic hyperplasia. Most cases present diandric-monogynic triploid genome [1, 3, 5, 6, 19].

Clinical presentations are vaginal bleeding, missed or incomplete abortion in the late first or early second trimester, normal to mild elevated serum hCG ($<100 \times 10^3$ mIU/mL), and a focal cystic change in the placenta on ultrasound. Fetal tissues or gestational sac can be seen [19].

Genetically, PM has a triploid diandric-monogynic genome with karyotype of 69XXY (70%), 69XXX (27%), and 69XYY (3%) (Fig. 13.1) [1, 3, 19].

Grossly, hydropic vesicles admixed with normal placental tissue are characteristic features (Fig. 13.4). Gestational sac, abnormal fetus, and normal fetus might be found.

Histological features are hydropically enlarged villi with occasional central cistern and oval or irregular outline, called "fjord-like" or "scalloping" (Fig. 13.5a). Trophoblastic stromal inclusions are commonly found and the result of villous irregular shape (Fig. 13.5b). Circumferential trophoblastic hyperplasia is not conspicuous; however, focal mild syncytiotrophoblastic hyperplasia called "knuckles" or "sprouts"

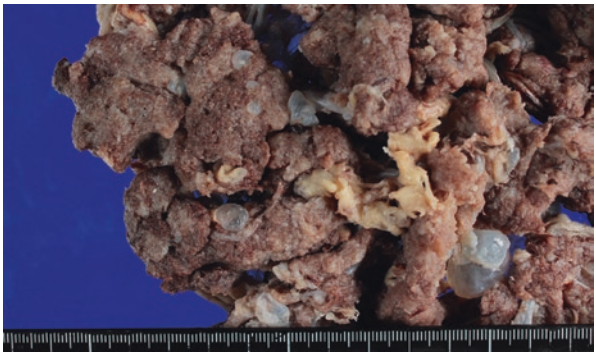

Fig. 13.4 Gross appearance of partial hydatidiform mole, 25 weeks of gestation with triploidy revealed by DNA genotyping. Scattered hydropic vesicles are seen in normal placental villi

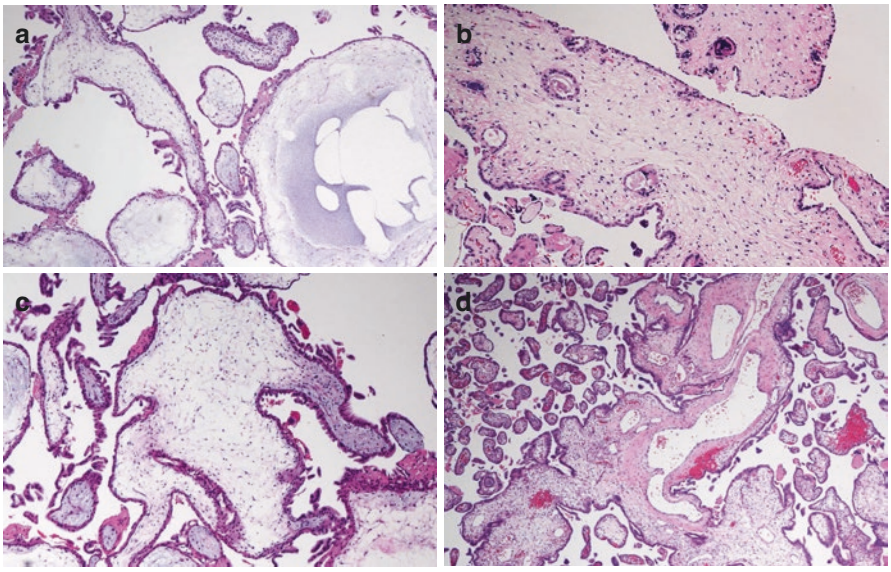

Fig. 13.5 Histologic appearance of partial hydatidiform mole. (**a**) A mixed population of villi with shape differences in villous size. (**b**) Trophoblastic inclusions and villous stromal fibrosis. (**c**) "Fjord-like" irregular-shaped villi with predominantly mild syncytiotrophoblastic proliferation like "sprouts". (**d**) Pseudoangiomatous change showing dilated vessels in the villous stroma

is a characteristic feature (Fig. 13.5c). Cytologic atypia is minimal to mild. Villous stroma sometimes contains pseudoangiomatoid change (Fig. 13.5d). Stromal fibrosis and nucleated red blood cells in villous stromal vessels, which are less common, but present, in CM, are commonly found in PM [1, 3, 15, 19].

The prevalence of persistent GTD is 0.5–5% in PM, especially in the case of invasive PM [16, 17]. The risk of developing choriocarcinoma is 0–0.5% [1, 3, 20]. Differentiating PM from CM is important because CM has higher risk (15–29%) of persistent GTD. There are cases in which the morphological distinction between PM and CM, especially ECM, may be difficult because of inter-observer variation of commonly observed features, for example, edematous villi with irregular outline, mild trophoblastic hyperplasia, cistern formation, and trophoblastic pseudo-inclusions.

13.1.3 Invasive Hydatidiform Mole

Invasive hydatidiform mole is defined as CM or PM that invades the myometrium and/or uterine vessels (Fig. 13.6) [1, 21]. Clinically, these cases present with vaginal bleeding with persistent elevation of serum hCG after primary evacuation of a hydatidiform mole. Uterine perforation caused by invasive hydatidiform mole has been

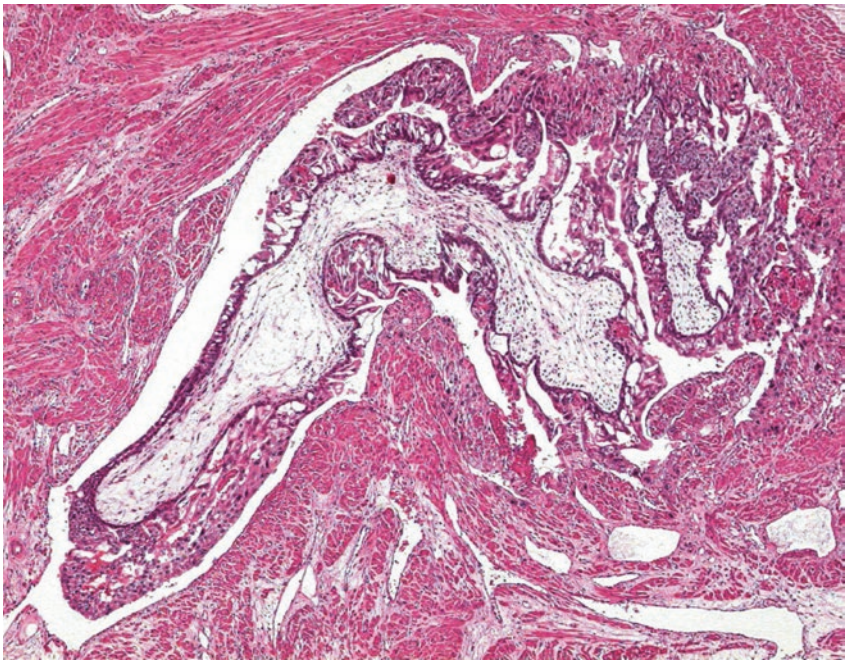

Fig. 13.6 Invasive complete hydatidiform mole. Molar tissue is identified in the myometrium

reported. Histologically, molar villi invading into myometrium are the diagnostic requirement. Histological features of CM or PM are same in the case of invasive hydatidiform mole. The finding of extravillous trophoblast (mainly intermediate trophoblast) without villi invading into superficial myometrium and maternal spiral artery is commonly seen in noninvasive moles and does not form the basis for a diagnosis of invasive hydatidiform mole. The incidence of invasive CM is higher than that of PM.

13.1.4 Ancillary Studies for the Diagnosis of Hydatidiform Mole

13.1.4.1 Immunohistochemistry

$p57^{KIP2}$ is an effective marker for differentiating CM from partial hydatidiform mole (PM) and hydropic abortus [3, 9–11]. $p57^{KIP2}$ is a cyclin-dependent kinase inhibitor encoded by the paternally imprinted and maternally expressed gene *CDKN1C* on chromosome 11p15.5. Due to its preferential expression from the maternal allele, the gene is silent in androgenic CM, PM, hydropic non-molar abortuses, and trisomies. These latter cases all show normal p57 protein expression pattern: positive nuclear staining in cytotrophoblast and villous stromal cells. In contrast, CM shows absent nuclear $p57^{KIP2}$ staining in cytotrophoblast and villous stromal cells because CM has only paternal genome, without any maternal genome (Fig. 13.7a, b). It is important to mention that the syncytiotrophoblast is negative and intermediate

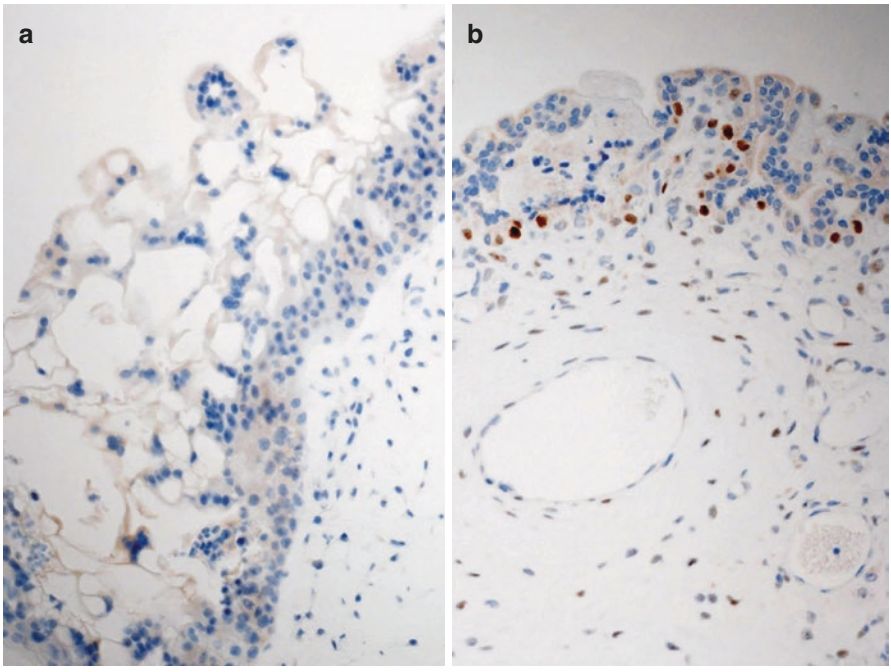

Fig. 13.7 Immunohistochemical staining of p57. (**a**) Complete hydatidiform mole. Absence of nuclear staining in cytotrophoblast and villous stromal cells. (**b**) Partial hydatidiform mole. Presence of nuclear staining in cytotrophoblast and villous stromal cells

trophoblast is positive for $p57^{KIP2}$ in all CM, PM, and non-molar villi. This may be a pitfall for the reviewer due to inexperience in recognizing the different types of trophoblast, especially the cytotrophoblast and intermediate trophoblast, resulting in inaccurate interpretation of immunohistochemical staining pattern. It is important to carefully note which cell types show nuclear-positive staining for $p57^{KIP2}$. Studies have revealed that p57 expression is highly correlated with DNA genotyping and it is a reliable marker for the diagnosis of CM. However, there are some exceptions of CM showing normal p57 pattern. For example, twin gestation with CM and normal fetus, rare CM of mosaic androgenic/biparental mosaic/chimeric gestations, and CM with retained maternal chromosome 11 are included. On the other hand, PM with p57-negative pattern based on loss of maternal chromosome rarely occurs [10]. Cell cycle proteins or proliferation markers (Ki-67, PCNA, ESF-1, CDK2, cyclin E) of molar pregnancies have also been studied and show variable results; however, none of them demonstrate the high sensitivity and specificity of p57 for use in routine diagnosis of hydatidiform mole [9].

13.1.4.2 Ploidy Analysis

Ploidy analysis has been used for decades in determining the number of haploid sets of chromosomes [9]. Karyotype analysis by chromosome G-band can also rule out chromosomal trisomy syndromes presenting with histological findings mimicking a molar gestation. The problems of ploidy analysis are: (1) it is unable to identify the paternal origin and cannot differentiate triploid PM from non-molar digynic triploidy; (2) fresh tissue is needed for karyotyping, whereas ploidy analysis can be performed on both fresh and fixed tissues; and (3) the rate of correct diagnosis of CM by FISH is only 38.5% because of technical difficulties.

13.1.4.3 Short Tandem Repeat (STR) Genotyping

Short tandem repeats (STRs) are prevalent, genetically stable, and repetitive noncoding DNA sequences. The number of repeats at each STR locus differs between individuals, and this feature is used for comparing the allelic profiles of maternal and molar tissue. Unstained tissue section from formalin-fixed paraffin-embedded tissue block(s) of maternal tissue and the chorionic villi are used to analyze the DNA genotype. After DNA extraction, PCR amplification using a commercially available kit is performed. By comparing the alleles of maternal and villous tissue at each STR locus, the presence and copy number of maternal and paternal alleles in the villi can be detected. With this technique, CM contains only paternal alleles of either homozygous or heterozygous pattern in at least two STR loci. One maternal allele and a duplicate quantity of one paternal allele at every STR locus are present in homozygous PM, and two different paternal alleles in addition to one maternal allele in at least two loci are detected in heterozygous PM [9–11].

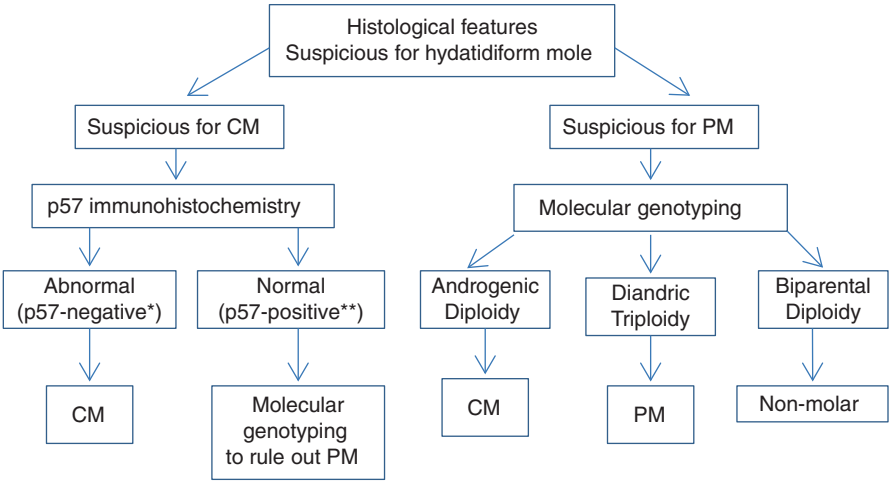

Fig. 13.8 Diagnostic algorithm for hydatidiform moles integrating immunohistochemistry and molecular genotyping [10]

Diagnostic algorithm of hydatidiform moles presented by Banet et al. is shown in Fig. 13.8 [10].

STR genotyping has the advantage of being able to precisely distinguish the paternal origin of DNA material in molar gestation. As such, it can accurately categorize the genotypes of molar pregnancies, for example, it can separate diandric triploid PM from non-molar digynic triploidy. In addition, genotyping does not require fresh tissue and can be performed retrospectively using formalin-fixed paraffin-embedded tissue.

Studies of DNA genotyping for CM by using STR loci detected by PCR have been reported in the USA [9, 11]; however, it has never been reported in Japan, while analysis of CM by using single-nucleotide polymorphism (SNP) genotyping, which is the measurement of genetic variations of SNPs, between maternal and villous tissues has been reported [22].

13.2 Gestational Trophoblastic Neoplasms

Gestational trophoblastic neoplasms (GTNs) include choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT). GTNs arise from different subtypes of trophoblast and have unique clinical, pathological, and genetic features. Clinical and histological features are summarized in Table 13.3.

Table 13.3 Diagnostic features of gestational trophoblastic tumors

Features	Choriocarcinoma	PSTT	ETT
Age	Reproductive age (ave. 30 y.o.)	20–63 y.o. (ave. 30 y.o.)	15–48 y.o. (ave.36 y.o.)
Antecedent pregnancy	Term pregnancy	Term pregnancy	Term pregnancy
	CM		
Interval time from index gestation	A few months to 14 years (ave.: 2 months after term pregnancy and 13 months after CM)	2 weeks to 17 years (median: 12–18 months)	1–25 years (ave. 6.2 years)
Clinical presentation	Vaginal bleeding	Missed abortion	Vaginal bleeding
	Persistent GTD	Amenorrhea	
Pretreatment hCG (mIU/mL)	$>10 \times 10^3$	$<1 \times 10^3$	$<3 \times 10^3$
Gross appearance	Circumscribed or Invasive hemorrhagic mass	Expansile to infiltrative solid large mass	Expansile solid mass
Tumor location	Corpus	Corpus	Cervix, Lower uterine segment, Corpus
Tumor border	Infiltrative	Infiltrative	Pushing
Tumor cells and cytologic atypia	Villous IT, ST and CT with marked atypia	Implantation site type IT, sometimes enlarged cells with moderate to marked atypia	Chorionic type IT, with mild to moderate atypia
Tumor growth pattern	Prominent hemorrhage and necrosis, trimorphic pattern of all three types of trophoblast	Tumor cells split myometrial smooth muscle fibers at tumor periphery and replacing vascular wall	Sheets, nests and cords, Geographic necrosis, Colonizing mucosal surface epithelium
Hyaline-like material	Absence	Presence, sometimes	Prominent
Stroma	No intrinsic tumor stroma or vasculature	Intimately infiltrates muscle fibers	Presence of nearby decidualized stromal cells
Immunohistochemistry	hCG, hPL, Ki-67 labeling index of >90%	hPL Mel-CAM, hCG (focal), Ki-67 labeling index of <10%	p63, hPL (focal), MEL-CAM (focal), Ki-67 labeling index of >10%

PSTT placental site trophoblastic tumor, *ETT* epithelioid trophoblastic tumor, *ave.* average, *y.o.* years old, *CM* complete hydatidiform mole, *GTD* gestational trophoblastic disease, *CT* cytotrophoblast, *ST* syncytiotrophoblast, *IT* intermediate trophoblast

13.2.1 **Choriocarcinoma** (Fig. 13.9a–d)

Choriocarcinoma is defined as a malignant tumor which consisted of mononuclear intermediate trophoblast or cytotrophoblast and multinucleated syncytial trophoblast without villi [1, 3, 5, 9].

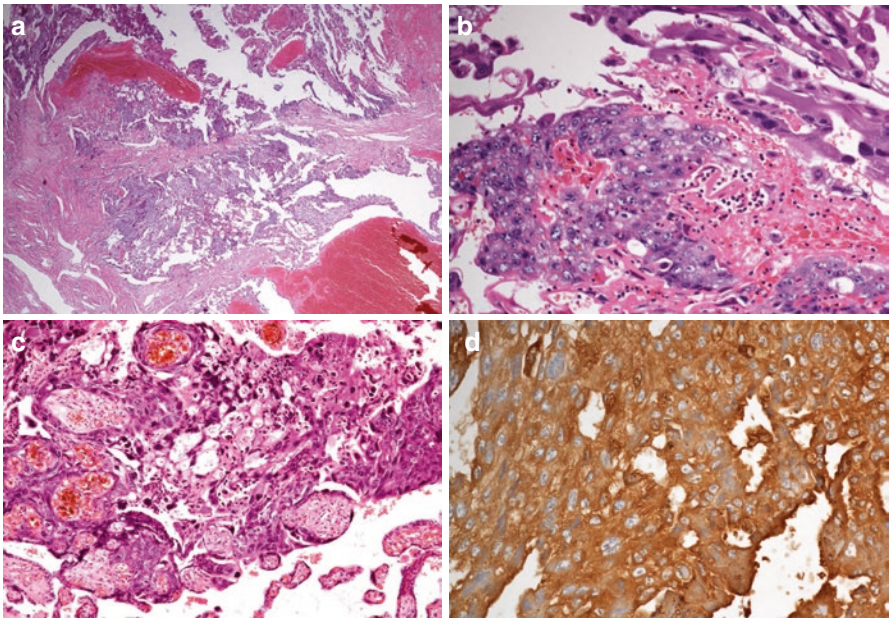

Fig. 13.9 Histologic appearance of choriocarcinoma. (**a**, **b**) The tumor cells invade into the myometrium with hemorrhage and necrosis. High-power view shows biphasic atypical trophoblast consisting of syncytial trophoblast (**b**, right upper side) and intermediate trophoblast or cytotrophoblast (**b**, left lower side). (**c**) Intraplacental choriocarcinoma in term placenta. Biphasic atypical trophoblast and nonneoplastic villi in the background. The fetus presented severe anemia caused by fetomaternal transfusion. (**d**) Immunohistochemical staining of hCG

Incidence of choriocarcinoma is 2–7/100,000 pregnancies in the USA and Europe and higher in Asia with 5–63/100,000 pregnancies. These rates are 1/20–1/40 of hydatidiform mole. In Japan, the incidence of choriocarcinoma as well as hydatidiform moles is decreasing. The risk of complete hydatidiform mole (CM) progress to choriocarcinoma is 2–3% and that of partial hydatidiform mole (PM) is 0–0.5%. Antecedent pregnancies include CM in 50%, missed abortion in 25%, and term pregnancy in 25% [1, 3, 5, 6]. Histological diagnosis of postmolar choriocarcinoma has been less common because treatment is administered based on only serological and imaging studies before hysterectomy. Interval time from index gestation is a few months to 14 years (average after normal pregnancy, 2 months; after CM, 13 months) [1, 3, 5]. Rarely intraplacental or in situ choriocarcinoma develops in full-term placentas with occasional concurrent metastatic disease. Marked fetal anemia caused by fetomaternal transfusion based on intraplacental choriocarcinoma has been reported [23].

Choriocarcinoma occurs in women of reproductive age with average of 30 years of age. Most common symptoms are vaginal bleeding and/or extrauterine hemorrhage caused by metastasis. Marked elevation of serum hCG, more than 10×10^3 mIU/mL, is always present. Persistent gestational trophoblastic disease

progressing to choriocarcinoma after CM is detected by persistent elevation of serum hCG [1, 3, 5].

Grossly, the tumor is typically a bulky, destructive red mass with prominent hemorrhage and necrosis. The tumor invades into the myometrium and occasionally multiple tumor nodules exist. In the case of choriocarcinoma after an ectopic pregnancy, tumor may develop in the extrauterine adnexa. Even in metastatic sites, marked hemorrhage and necrosis are common. Intraplacental choriocarcinoma presents typically with white nodules simulating placental infarction and may be quite difficult to recognize as a tumor [23].

Microscopically, choriocarcinoma presents with marked hemorrhage and necrosis in the center of the tumor, and tumor cells exist at the peripheral part of the mass and invade into the myometrium and vessels. The tumor cells are composed of malignant syncytiotrophoblast and intermediate trophoblast or cytotrophoblast with striking cytologic atypia (Fig. 13.9a, b) [1]. This pattern is called "biphasic pattern," although trimorphic proliferation of all three trophoblasts commonly occurs. Syncytiotrophoblast is a hyperchromatic, multinucleated cell, with polymorphic, relatively broad, and eosinophilic cytoplasm. Intermediate trophoblast or cytotrophoblast is a hyperchromatic mononuclear cell with prominent nucleoli and frequent mitotic figures showing solid and sheet-like proliferation patterns [1, 3, 9]. Normal or abnormal chorionic villi are absent, except in the case of intraplacental choriocarcinoma (Fig. 13.9c) [23].

Immunohistochemically, all tumor cells strongly express cytokeratin, hCG (Fig. 13.9d), and high rate of Ki-67 labeling index with more than 90% positivity. Intermediate trophoblast is positive for Mel-CAM (CD146) and MUC-4 [1, 9].

Differential diagnoses include exaggerated placental site, invasive CM, PSTT, ETT, and poorly differentiated carcinoma with trophoblastic differentiation. Exaggerated placental site is a nonneoplastic implantation site change consisting of intermediate trophoblast. It is occasionally similar to choriocarcinoma, especially in curettage specimens. The cells of exaggerated placental site have mild to moderate cytologic atypia and rare mitosis. Ki-67 labeling index of less than 1% in exaggerated implantation site reaction is helpful to differentiate it from choriocarcinoma. Invasive CM can be excluded when hydropic villi are not found; however, distinguishing these entities can become problematic when only scant tissue samples are available [1, 9].

Untreated choriocarcinoma frequently metastasizes to the vagina, lung, liver, brain, and kidney. Scores categorizing the severity of disease based on FIGO/WHO classification system (Table 13.4) including various factors from the history and clinical examination give a combined score that predicts the potential of resistance to single-agent chemotherapy [1, 24]. Most patients with GTN following a hydatidiform mole have a FIGO/WHO score of 0–6, indicating low risk of developing GTN resistant to single-agent chemotherapy with methotrexate or actinomycin D, and high-risk GTN (score $\geq$7) cases are considered clinically as choriocarcinoma. Patients with high-risk score or resistance with single-agent therapy are treated with combination-agent chemotherapy, generally methotrexate, actinomycin D, and etoposide [3]. Over 90% of patients are cured by combined and sequential chemotherapy [1, 3, 5].

Table 13.4 FIGO/WHO scoring system of prognostic and predictive parameter for trohpblastic tumours

Prognostic factor	0	1	2	4
Age	<40	≥40		
Antecedent pregnancy	Mole	Abortion	Term pregnancy	
Interval, months from index gestation	<4	4–6	7–12	>12
Pretreatment hCG (mIU/mL)	$<10^3$	10^3–10^4	10^4–10^5	$>10^6$
Largest tumor size, including uterus	<3 cm	3–5 cm	>5 cm	
Site of metastasis	Lung	Spleen, kidney	GI tract	Brain, liver
Number of metastasis		1–4	5–8	>8
Previous failed chemotherapy			Single agent	Two or more agents
Total score				

Low-risk, score ≤6; high-risk, score ≥7; *hCG* human chorionic gonadotropin

13.2.2 Placental Site Trophoblastic Tumor (PSTT)

Placental site trophoblastic tumor (PSTT) is a rare trophoblastic tumor consisting of neoplastic implantation site intermediate trophoblast [1, 25, 26]. The incidence of PSTT in gestational trophoblastic disease is 0.23–3%. The patient's age in PSTT ranges from 20 to 63 years, with mean age of 30 years. In most cases, the antecedent pregnancies are term pregnancies, and interval time to diagnosis for the index gestation ranged from 2 months to 17 years (mean, 12–18 months). The fetus of antecedent pregnancy of PSTT tends to be overwhelmingly female (M:F = 2:11). The most common clinical presentation is vaginal bleeding. Pretreatment serum hCG is usually less than 1000 mIU/mL. Eighty percent of reported cases are FIGO stage I, and 10–20% of cases are FIGO stage II with metastasis to the lung, adnexa, pelvic lymph node, and parametrium.

Cytogenetically, the absence of the Y chromosome in PSTT with a haploid pair of X chromosomes has been reported, and the paternal X chromosome is considered to be related to tumorigenesis of PSTT [27].

Macroscopically, PSTT presents as an endomyometrial nodular mass of 1–10 cm in diameter. The tumor is relatively well circumscribed and white-tan to light yellow in color and invades into the myometrium in 50% of the cases. Focal hemorrhage and necrosis are present in nearly half of the cases (Fig. 13.10a).

Histologically, the tumor cells have an infiltrative growth pattern with cords or sheet-like aggregates (Fig. 13.10b). The most charachteristic features of PSTT are infiltrative tumor cells splitting individual myometrial smooth muscle fibers at the peripheral part of the tumor and replacing the vascular wall of myometrial vessels. These cells are neoplastic implantation site intermediate trophoblast with abundant eosinophilic or clear cytoplasm and marked hyperchromatic, convoluted large nuclei. Syncytiotrophoblast with multinucleated cells is commonly scattered; however, mononuclear monotonous intermediate trophoblast forms the vast majority of the tumor. Mitotic activity is relatively low ranging from 2 to 4 per 10

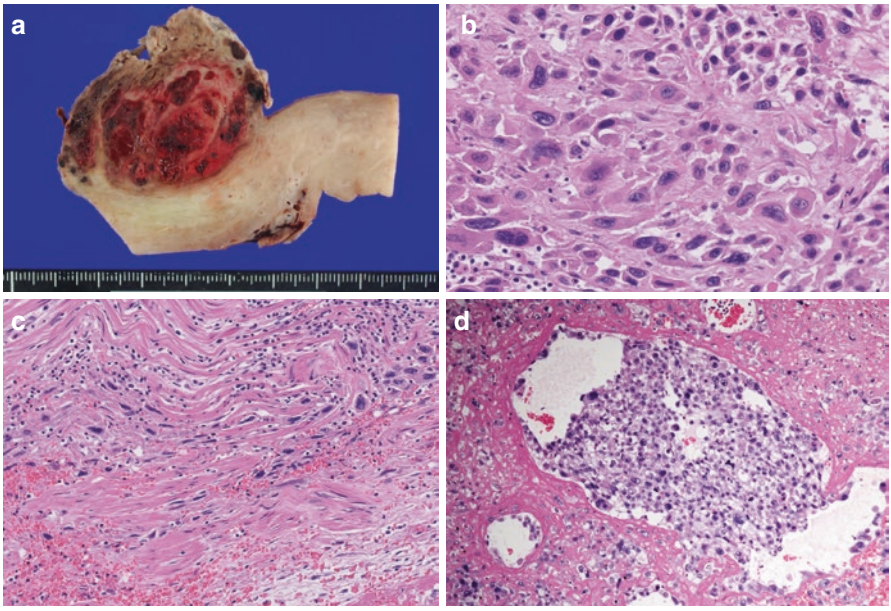

Fig. 13.10 Placental site trophoblastic tumor (PSTT). (**a**) Gross appearance of PSTT. Discrete solid mass in the endomyometrium presenting focal hemorrhage. (**b**) Sheet of atypical intermediate cells with eosinophilic abundant cytoplasm and large convoluted nuclei. (**c**) Tumor cells infiltrate and split existing smooth muscle fibers at the tumor periphery. (**d**) Tumor cells replacing the vascular wall of the myometrial vessel

high-power fields; however, cases with higher mitotic activity are found occasionally. Hemorrhage and necrosis are not uncommon [9, 25, 26].

Immunohistochemically, tumor cells are diffusely positive for Mel-CAM (CD146) (Fig. 13.11a), hPL, MUC-4, and HLA-G and negative for p63 (Fig. 13.11b). Expression of hCG (Fig. 13.11c) and inhibin is focal (Fig. 13.11a, b). Ki-67 labeling index is 10–30% in most of the cases (Fig. 13.11d) [28].

Differential diagnoses include exaggerated placental site, choriocarcinoma, ETT, epithelioid leiomyoma/leiomyosarcoma, and poorly differentiated carcinoma. Differentiation from exaggerated placental site is the most frequent problem on routine pathological diagnosis. Exaggerated placental site is also composed of implantation site-type intermediate trophoblast with cytomorphological similarities with PSTT. However, exaggerated placental site does not present as a nodular lesion with increased mitotic activity. Ki-67 labeling index is an effective marker with 0–2% positivity in exaggerated placental site and 10–30% in PSTT.

Most patients are cured by hysterectomy [3]. The previously described scoring systems are not used for PSTT. The recurrence rate is 25–30% and half of these patients may die of PSTT. Histological parameters of worse prognosis are tumor cells with clear cytoplasm, depth of invasion, tumor size, necrosis, and high mitotic count (>5 per 10 HPF) [26].

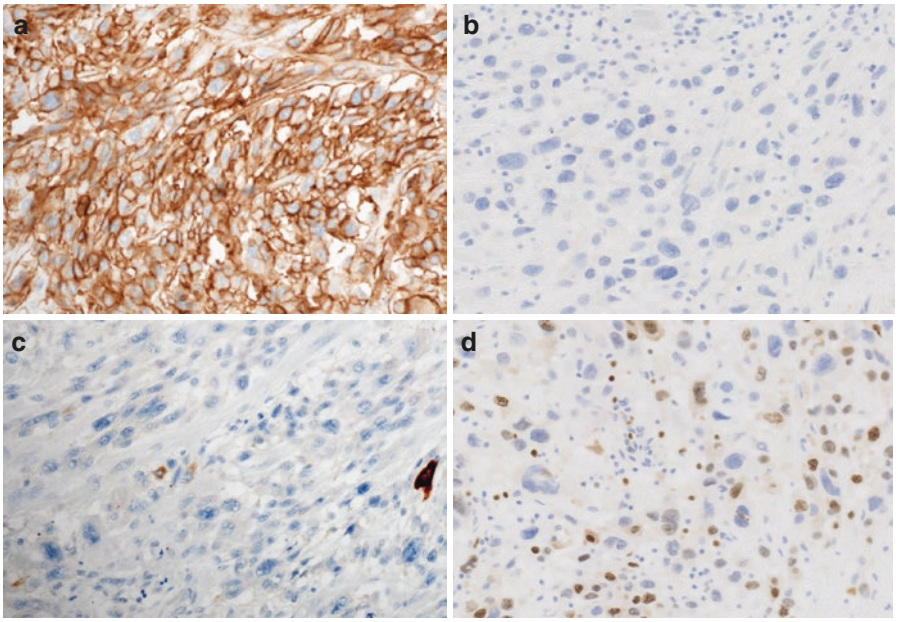

Fig. 13.11 Immunohistochemical staining for PSTT. (**a**) Mel-CAM (CD146). The tumor cells diffusely express. (**b**) p63, negative. (**c**) hCG. Most of the tumor cells are negative with scattered positive cells. (**d**) Ki-67 labeling index is about 30%

13.2.3 Epithelioid Trophoblastic Tumor

Epithelioid trophoblastic tumor (ETT) is defined as a trophoblastic tumor consisting of neoplastic intermediate trophoblast arising from the chorion laeve [1, 9, 29, 30]. The patient ages range from 15 to 48 years (mean of 36 years). Common symptom is vaginal bleeding. In most cases, antecedent pregnancy is term pregnancy, and interval time ranges from 1 to 1.5 years with average of 6.2 years. Serum hCG level shows only a mild to moderate elevation of less than 2500 mIU/mL. Thirty-five percent of reported cases already had metastases at the time of initial diagnosis.

Genetically, the absence of a Y chromosome with a haploid pair of X chromosomes in ETT as well as PSTT has been reported [31].

ETT is distributed as follows: 30% in the uterine corpus, 50% in the fundus or cervix, and 20% in extrauterine sites including the small intestine and lung. The tumor size is 0.5–4 cm in diameter, forming discrete nodule or a cystic hemorrhagic mass. The tumor cells deeply invade into the myometrium. The cut surface of the tumor is solid and has white-tan to brown color with various amounts of hemorrhage and necrosis [1, 9, 29, 30].

Histologically, ETT is a nodular lesion of medium-sized mononuclear tumor cells arranged in nests or cords to a sheet-like appearance. The tumor cells are relatively uniform with small, round to oval nuclei, eosinophilic or clear cytoplasm, and

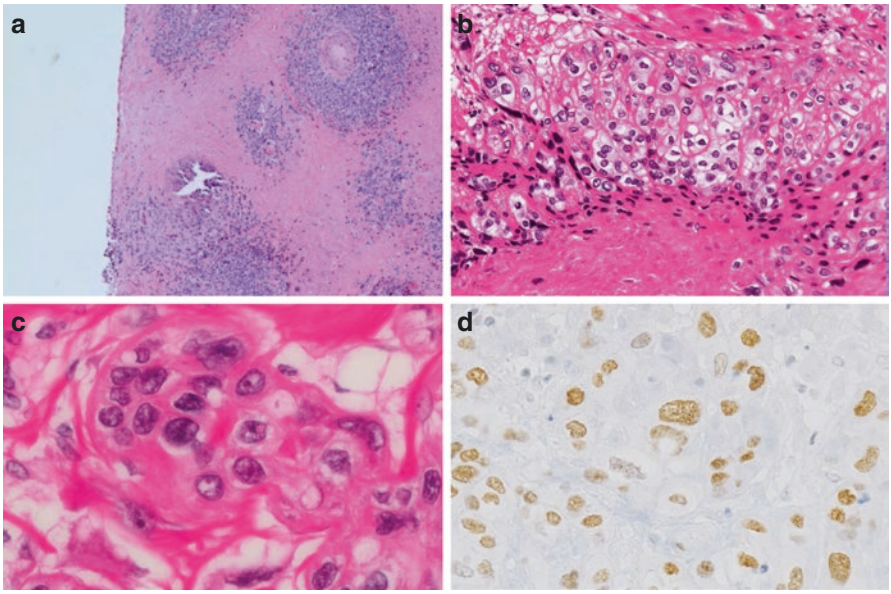

Fig. 13.12 Epithelioid trophoblastic tumor. (**a**) The tumor is characterized by geographic necrosis. (**b**) A nest of tumor cells with a relatively uniform populations of mononuclear intermediate trophoblastic cells with necrosis and fibrinoid deposition. (**c**) The tumor cells with eosinophilic to clear cytoplasm and moderate nuclear atypia. Deposition of hyaline-like material in the background. (**d**) The tumor cells express nuclear positivity for p63. (**b**, **d**, courtesy of Dr. Takako Kiyokawa; **c**, courtesy of Dr. Yuichiro Sato)

distinct cell membranes. Cytologic atypia is mild to moderate and mitotic rate is 0–9/10 HPF. Hyaline-like material in the center of the tumor or between tumor cells is the most characteristic feature. Extensive geographic necrosis is also a common feature (Fig. 13.12a–c). Histological findings are occasionally similar to squamous cell carcinoma. In cases of cervical involvement of ETT, a cervical mucosal lesion simulating high-grade squamous intraepithelial lesion is often seen [1, 9, 29, 30].

Immunohistochemistry is very helpful in differentiating ETT from its mimics, squamous cell carcinoma, PSTT, and placental site nodule [9, 28]. ETT is diffusely positive for p63 (Fig. 13.12d), HLA-G, and inhibin-alpha. Mel-CAM (CD146) and hPL are expressed focally, whereas PSTT is p63 negative and shows diffuse strong positive with Mel-CAM and hPL. Squamous cell carcinoma is positive for p63 and negative for trophoblastic markers. ETT shows Ki-67 labeling index higher than 10%, whereas Ki-67 labeling index of placental site nodule is less than 10%.

The main treatment for ETT is surgical, and the FIGO/WHO scoring system is not used for ETT. The prognosis of ETT is similar to that of PSTT. The rate of metastasis is 25%, and 10% of these patients may die of disease. The survival rates are 100% in patients without metastasis and 50–60% in patients with metastasis [3]. Histological predictor of a worse prognosis is higher mitotic counts (> 6/10 HPF) [1, 29, 30].

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