11 Markers and Immunoprofile of Tumors of Female Reproductive Organs

Contents

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11.1 Diagnostic Antibody Panel for Tumors of the Vulva and Vagina

Cytokeratin profile, p40, p63, CEA, p16, Ki-67, HPV, Desmin, Myogenin, and melanoma markers.

11.2 Diagnostic Antibody Panel for Epithelial Tumors of the Uterine Cervix

Cytokeratin profile, p40, p63, CEA, PAX-8, PAX-2, p16, p53, Ki-67, HPV, and Steroid hormone receptors [1].

11.3 Diagnostic Antibody Panel for Epithelial Tumors of Uterine Corpus, Fallopian Tube, and Uterine Ligament

Cytokeratin profile; CEA; PAX-8; p16; HNF-1β; WT-1; Steroid hormone receptors (ER, PgR); DNA mismatch repair proteins (MLH1, PMS2, MSH2, MSH3, MSH6); p53; and Ki-67.

11.4 Diagnostic Antibody Panel for Uterine Mesenchymal Tumors

Smooth muscle markers (Actin, Smoothelin, Caldesmon, Calponin); IFTIM (CD225); CD10; and Steroid hormone receptors (ER, PgR).

11.5 Diagnostic Antibody Panel for Gestational Trophoblastic Disease

Cytokeratin profile, PLAP, human leukocyte antigen G (HLA-G), human placental lactogen (hPL), GATA-3, and Inhibin.

11.5.1 p16

p16 (CDKN2A)		
Expression pattern: Nuclear/cytoplasmic		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– HPV-associated oropharynx and uterine cervix squamous cell carcinoma – Atypical lipomatous tumors and liposarcoma	Endometrial serous carcinoma, clear cell carcinoma, melanocytic nevi and melanoma, adenoid cystic carcinoma, leiomyosarcoma, and smooth muscle tumors of uncertain malignant potential	
Positive control: Cervical squamous cell carcinoma		

Diagnostic Approach p16 (also known as INK4a or cyclin-dependent kinase inhibitor 2A) is a tumor suppressor protein encoded by the $p16^{INK4a}$ (CDKN2) suppressor gene. p16 inhibits the cyclin-dependent kinases (4 and 6) involved in cell cycle regulation and progression (G1 to S). p16 plays a role in the pathogenesis of different malignancies. The expression of p16 is regulated by the activity of the retinoblastoma gene (Rb), which in turn is affected by the E7 oncogene of the HPV gene. p16 is overexpressed in HPV-associated intraepithelial dysplasia and squamous cell carcinomas of different origins, including vulvar, vaginal, and cervical squamous cell carcinoma in addition to oropharynx carcinoma. In routine immunohistochemistry, p16 reveals cytoplasmic and nuclear staining patterns and the intensity of the stain correlates with the grade of HPV infection and the grade of associated dysplasia. The so-called block staining pattern is characteristic for HPV-associated high-grade dysplasia (Fig. 11.1) and HPV-associated squamous cell carcinoma. p16 is

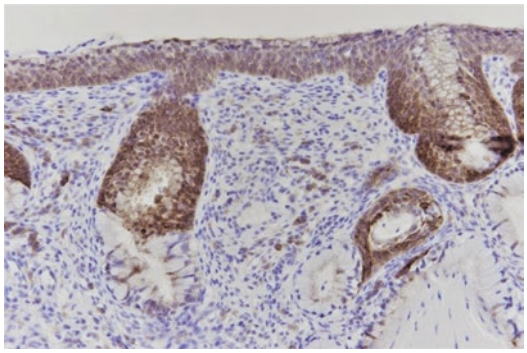


Fig. 11.1 Cervical biopsy with HPV-associated high-grade dysplasia. Dysplastic cells exhibit a strong block-like p16 expression

usually negative in HPV-independent squamous cell carcinomas, which in turn are frequently positive for p53.

p16 is also highly expressed in serous uterine carcinoma and is a helpful marker that labels the cells of serous tubal intraepithelial carcinoma (STIC) [2].

p16 is also a helpful marker to discriminate between benign and malignant adipocytic tumors and between benign nevi and malignant melanocytic tumors (see related chapters) [3, 4].

PAX-8 PAX-8 is a transcriptional factor involved in the fetal development of the brain, eye, thyroid tissue, kidney, and upper urinary system, as well as the Müllerian organs. PAX-8 is one of the best markers for endometrial adenocarcinoma and a subset of endocervical adenocarcinomas. This marker is listed in detail in the following chapter (see Chap. 12).

11.5.2 Hepatocyte Nuclear Factor-1 β

Hepatocyte nuclear factor-1 β (HNF-1 β) is a member of the hepatocyte nuclear factor family regulating the growth and differentiation of hepatocytes and cells of the biliary system. The expression of different hepatocyte nuclear factors is not restricted to the liver but is also variously found in other organs, including the pancreas, kidney, prostate, and female genital system [5]. HNF-1 β is used in diagnostic immunohistochemistry to differentiate between different types of ovarian and endometrial carcinomas. The strong nuclear HNF-1 β expression is characteristic for both endometrial and ovarian clear cell carcinomas but is usually negative in reactive lesions with clear cell appearance such as clear cell metaplasia and Arias-Stella phenomenon [6]. However, we must consider that a focal weak to moderate HNF-1 β expression can also be found in other endometrial and ovarian carcinoma types, such as endometrioid and serous carcinomas [7]. Additionally, a different HNF-1 β expression intensity is also found in other carcinomas of different origins, including colorectal, pancreatobiliary, prostatic, and renal cell carcinomas.

11.5.3 Phosphatase and Tensin Homolog

Phosphatase and **ten**sin homologue (PTEN) is a tumor suppressor gene located on 10q23 and encoding a widely expressed enzyme in mammalian cells that catalyzes the dephosphorylation of the 3'phosphate of the inositol ring, which is an essential reaction that causes the inhibition of the protein kinase (AKT) signaling pathway involved in the regulation of apoptosis. Deletions or mutations that inactivate the PTEN gene cause the inhibition of the apoptotic cascade and increase cell proliferation, mainly by the upregulation of the mammalian target of rapamycin (mTOR). Inactivating mutations within the PTEN are commonly seen in different human neoplasia such as urogenital, breast, and lung carcinomas in addition to melanoma and glial tumors [8]. The immunohistochemical staining of PTEN (cytoplasmic or nuclear stain pattern) is a simple way to detect the loss of this enzyme but may be difficult if the immunohistochemistry signal in normal glands is weak; careful titration of the primary antibody is key. The loss of PTEN expression is found in 35–55% of endometrioid carcinoma and in up to 65% of atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia, which indicates that the loss of PTEN is not a specific marker of malignant transformation [9, 10]. Normal proliferative endometrium usually shows strong PTEN expression. The loss of PTEN expression is also found in a subset of endometrioid ovarian carcinoma (~20%), high-

grade serous carcinoma, and clear cell carcinoma.

Prostatic adenocarcinoma is also commonly associated with PTEN loss (see markers of prostatic carcinoma, Sect. 13.1) [8]. PTEN mutations with a loss of protein expression are also found in a subset of thyroid adenomas and different breast carcinoma types in addition to primary glioblastoma but rare in secondary glioblastoma.

11.5.4 Steroid Hormone Receptors

Both estrogen and progesterone receptors, in addition to androgen receptors, were discussed in detail with the markers of breast tumors. Endometroid adenocarcinoma and serous endometrial carcinoma are sex hormone-dependent tumors, and the expression of estrogen and progesterone is characteristic for both carcinoma types in various degrees [11]. More than 90% of grade 1 and 2 and ~ 50% of grade 3 endometroid carcinoma are positive for both hormone receptors and generally endometroid carcinomas, with the strong expression of steroid hormone receptors rarely associated with the overexpression of p53. The myometrium is also a target tissue for steroid hormone receptors; accordingly, the majority of uterine leiomyomas and leiomyosarcomas are positive for estrogen or progesterone receptors or for both. This characteristic feature can be used to differentiate between uterine and soft tissue leiomyosarcoma [12]. Squamous cell carcinoma and adenocarcinoma of the uterine cervix usually lack the expression of both receptors [13].

11.5.5 Mismatch Repair Proteins, Microsatellite Instability, and Molecular Classification of Endometrioid Carcinoma

Mismatch repair proteins and detection methods, including immunohistochemistry on paraffin fixed tissue, are discussed in detail in Chap. 35. In gynecological pathology, the analysis of the mismatch repair proteins is essential for the diagnosis and classification of uterine and ovarian carcinomas.

Microsatellite instability (MSI-H/MMRd) is detected in up to 30% of endometrial and ovarian endometrioid carcinomas as well as clear cell ovarian carcinoma and can show different expression patterns of the MMR proteins.

- Normal nuclear expression of the four MLH1, PMS2, MSH2, and MSH6 proteins. This normal immunohistochemical pattern indicates no evidence of mismatch repair deficiency.
- Loss of MLH1 and PMS2: This type is found in 90–95% of endometrioid carcinomas with microsatellite instability. The majority of these carcinomas are associated with the hypermethylation of the MLH1 promoter region and are considered sporadic tumors. The absence of promoter hypermethylation is found in 3–5% of endometrioid carcinomas and is generally due to a germline mutation/Lynch syndrome.
- Single loss of PMS2 (or rarely MLH1): This type is mostly associated with germline mutations in PMS2/MLH1 genes with a high probability for Lynch syndrome.
- Loss of MSH2 and MSH6: Mainly caused by a germline mutation in the MSH2 gene and the evaluation of EPCAM expression is indicated.
- Single loss of MSH6: mostly caused by germline mutations in the MSH6 gene, usually with a high probability for Lynch syndrome.

Four distinct molecular groups of endometrioid carcinomas of the endometrium are described and have different prognoses and therapy management: [14, 15]

- Group 1: ultramutated carcinomas with mutations in the exonuclease domain of the DNA polymerase epsilon (POLE) gene. This gene is responsible for a low mutation rate in DNA replication. This group includes all of POLE mutated endometrium carcinomas regardless

of mismatch repair status or p53 mutations and is usually associated with a good prognosis.
- Group 2: hypermutated carcinomas with microsatellite instability (MSI-H/MMRd). This group is associated with an intermediate prognosis.
- Group 3: carcinomas with low-copy-number alterations. These carcinomas are microsatellite stable and lack TP53 or POLE mutations. These carcinomas are classified as carcinomas with a nonspecific molecular profile and usually have an intermediate prognosis.

Group 4: carcinomas with high-copy-number alterations and recurrent TP53 mutations with a strong p53 expression. These carcinomas are classified as serous-like carcinomas and have a poor prognosis (see Chap. 36).

11.5.6 p53

p53 is a tumor suppressor protein that binds to DNA, inducing the synthesis of the p21 protein. Mutations within the TP53 gene cause the abnormal expression or the absence of the p53 protein, resulting in an uncontrolled proliferation of the involved cells. The p53 expression pattern is an important criterion for the classification of endometrial and ovarian carcinomas and the detection of premalignant tubal lesions (serous tubal intraepithelial carcinoma, STIC; see Fig. 11.2). p53 is listed in detail in Chaps. 33, 36.

11.5.7 Interferon-Inducible Transmembrane Protein-1

Interferon-inducible transmembrane protein-1 (IFITM-1, clustered as CD225) is a member of

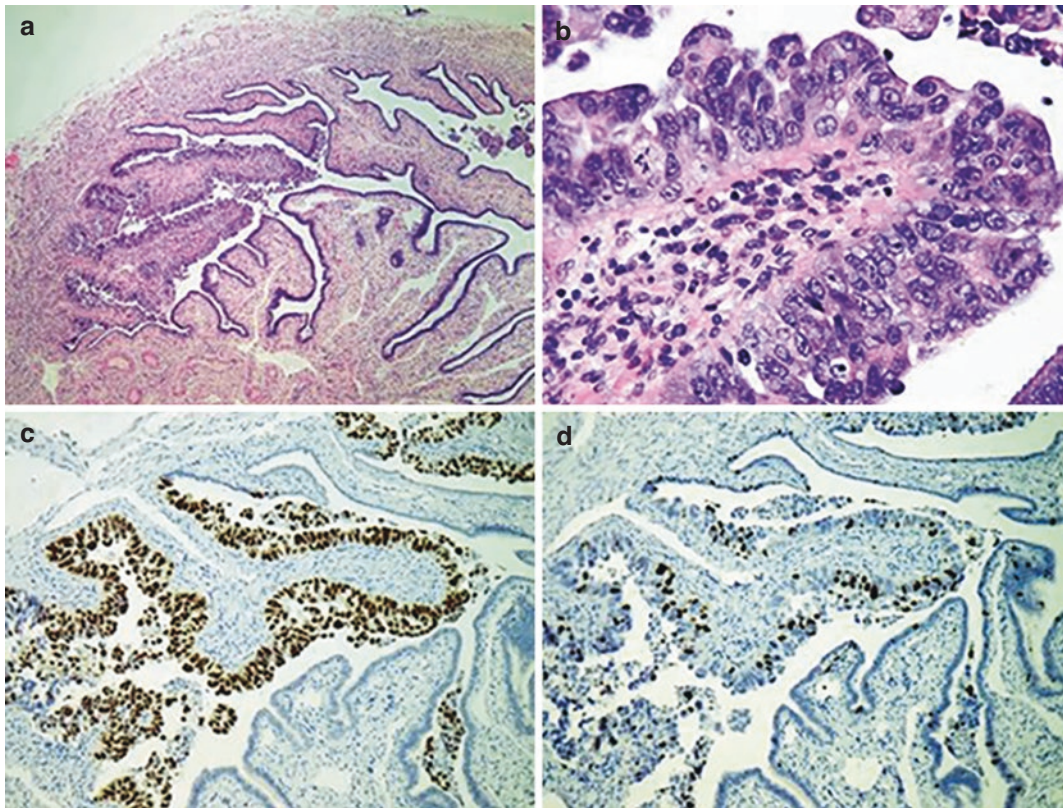


Fig. 11.2 Serous tubal intraepithelial carcinoma (STIC). (**a**, **b**) H&E 40X and 200X showing fallopian tube with marked atypia of tubal epithelium, (**c**) same section with strong diffuse nuclear p53 accumulation, and (**d**) Ki-67 expression in ~15% of epithelial cells

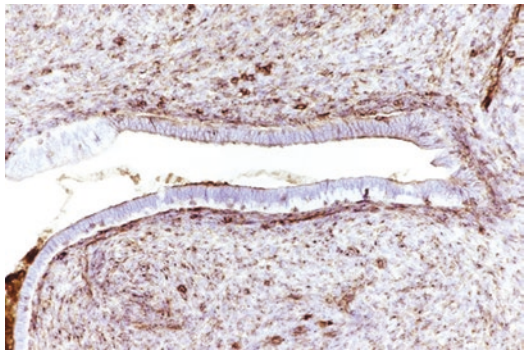


Fig. 11.3 Low-grade endometrial stromal sarcomas with IFITM-1 expression in tumor cells

the IFITM family functioning as a surface receptor regulating the CD19 phosphorylation. IFITM-1 is also described as a novel marker for endometrial stromal differentiation and is considered a specific marker for endometrial stromal cells. IFITM-1 is a sensitive marker for endometrial stromal nodules and low-grade endometrial stromal sarcomas but is negative in uterine smooth muscle tumors (Fig. 11.3) [16, 17].

11.5.8 GATA-3

GATA-3 is a transcription factor listed in detail in other chapters (see Chap. 10 and Sect. 12.2). In uterine, ovarian, and testicular germ cell tumors, GATA-3 is also used as a pan-trophoblastic marker that labels cytotrophoblasts, intermediate trophoblasts, and syncytiotrophoblasts.

11.5.9 Human Placental Lactogen

Human placental lactogen (hPL), also known as human chorionic somatomammotropin, is a placental hormone involved in the regulation of maternal and fetal metabolism and expressed by the placental syncytiotrophoblasts. In routine immunohistochemistry, hPL is a marker for intermediate trophoblasts, syncytiotrophoblastic cells of choriocarcinoma, placental site trophoblastic tumors, and exaggerated placental sites and usually negative in placental site nodules and epithelioid trophoblastic tumors.

Immunoprofile of tumors of the uterine cervix, uterine corpus, and fallopian tube				
Tumor type	+ in >90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in <10% (−)
A. Tumors of the vulva and vagina				
Paget disease of the vulva:	CK7, EMA (MUC1), TRPS-1, CEA, androgen receptors	ER, GCFP-15		CK5/6/14, CK20
Squamous cell carcinoma, HPV-associated:	**CK5/6**, CK19, p63, **p16**			CK7, CK20
Squamous cell carcinoma HPV-independent:	CK5/6, CK18, CK19, p63	p53		CK7, CK20, **p16**
Mucinous carcinoma, gastric type:	CK7, CDX-2, PDX-1, CEA		PAX-8, CK20, p53	p16, ER
Mucinous carcinoma, intestinal type:	CK20, CDX-2, PDX-1	SATB-2	CK7	ER
Bartholin gland carcinoma: – Adenocarcinoma – Squamous cell carcinoma – Adenoid cystic carcinoma – Transitional cell carcinoma	See immunoprofile of similar carcinomas of other locations			
Adenocarcinoma of mammary type:	See immunoprofile of breast carcinoma			
Adenocarcinoma of skene gland type:	Pan-CK, NKX3.1, PSA			PAX-8

Clear cell carcinoma:	CK7, EMA, CEA	HNF1-β, Napsin A		CK20
Sebaceous carcinoma:	**Adipophilin**, EMA, androgen receptors	Perilipin, CK5/14, CK8/18, CK7, CK19, CD15, p16		CK20, CEA, S100
Angiomyofibroblastoma:	Desmin, ER, PgR		CD34	Actin
Fibroepithelial stromal polyp:	Desmin, ER, PgR		Actin, Myogenin	
Cellular angiofibroma:		CD34, ER, PgR		Actin, Desmin
Prepubertal fibroma:	CD34		ER, PgR	Actin, S100, Desmin
Superficial myofibroblastoma:	CD34, Desmin, ER, PgR			S100
Superficial angiomyxoma:	CD34			Actin, Desmin, S100
Deep aggressive angiomyxoma:	Desmin, actin, HMGA2	CD34, ER, PgR,	FXIIIa	Myogenin, MyoD1, Smoothelin, S100
Epithelioid sarcoma:	See miscellaneous soft tissue tumors			
Rhabdomyosarcoma:	See soft tissue rhabdomyosarcoma			
B. Tumors of the uterine cervix				
Squamous cell carcinoma, HPV-associated:	**CK5/6**, CK19, p63, p40, **p16**	CK14		CK7, CK20, ER, PgR
Squamous cell carcinoma HPV-independent:	**CK5/6**, CK19, p63, p40	p53		CK7, CK20, ER, PgR, **p16**
Mesonephric remnants/ hyperplasia/carcinoma	CD10, **GATA-3**	PAX-8, TTF-1		ER, PgR, p16
Endocervical adenocarcinoma, HPV-associated: – Usual type – Mucinous type (mucinous NOS, intestinal adenocarcinoma, signet ring cell adenocarcinoma, and stratified mucin-producing carcinoma)	**CK7**, CK8/18, CK19, **CEA**, EMA, **p16**	**PAX-8**	CK20, PDX-1[n], CDX-2[n], Vimentin	**ER**, PgR, CK5/6, p63, p40, WT-1, PAX-2[l], GFAP
Endocervical adenocarcinoma, HPV-independent, gastric type:	CK7, PAX-8	PDX-1	CK20, CDX-2, HNF1-β	PAX-2, ER, PgR, p16
Endocervical adenocarcinoma, HPV-independent, clear cell type:	CK 7, EMA, CA125, PAX-8, **HNF1-β**, AMACR	Napsin A, CD15, p16, Vimentin	AFP, CEA, p53, sox-2	ER, PgR, WT-1, GATA-3, mammaglobin, CK20, CD10
Endocervical adenocarcinoma, HPV-independent, mesonephric type:	CK5/6, CK7, CK8/18, GATA-3, PAX-2, PAX-8, EMA, CD10, CD15	CD10, p16, PAX-8, bcl-2, Vimentin	Androgen receptors, TTF-1, Calretinin, inhibin	PgR, ER, CK20, Napsin A, CEA, p53
Endometrioid adenocarcinoma of endocervix:	**CK7**, CK8, CK18, CK19, EMA, PAX-8, Vimentin,	**ER**, PgR, GFAP	p16, CD56, PAX-2	CK20, CK5/6, **CEA**, CDX-2
Serous carcinoma:	**CK7**, CK8, CK18, CK19, EMA, CA125, **p16**, **p53**, **PAX-8**, Vimentin, β catenin	**IMP3**	ER, PgR, sox-2, WT-1, Napsin A,	CK5/6, CK20, mammaglobin, HNF1-β
Adenosquamous carcinoma (glassy cell carcinoma):	CK7[c], PAX-8[c], CK5/6/14[b], p16			ER, PgR

Adenoid basal carcinoma:	CK5/14, CK7, p63, p40	p16		CD117
Neuroendocrine tumors: – NET G1[c] – NET[d] G2 – NET G3 and NEC (small cell carcinoma)[e]	Pan-CK, **CD56**, Insm-1, NSE, PGP9.5 Proliferation index (Ki-67) in: NET G1: < 2% NET G2: 3–20% NET G3: > 20% NEC G3: > 20%	**Synaptophysin**, **chromogranin**, SSTR2	TTF-1	CK7, CK20
C. Epithelial tumors of the uterine corpus				
Endometrioid adenocarcinoma:	**CK7**, CK8/18, CK19, **PAX-8**, **sox-17**, EMA, **Vimentin**, CA125	**ER**, PgR, GFAP	Mammaglobin, p16, CD56, p53	CK5/6, CK20, CEA, WT-1, **IMP3**, CDX-2[f]
Serous endometrial carcinoma:	**CK7**, CK8/18, CK19, EMA, CA125, **p16**, **p53**, **PAX-8**, **sox-17**, **IMP3**, Vimentin, β catenin Proliferation index (Ki-67): >75%		ER, PgR, sox-2, WT-1	CK5/6, CK20, mammaglobin, HNF1-β, PTEN
Clear cell carcinoma:	CK 7, EMA, CA125, **PAX-8**, **HNF1-β**, **sox-17**, **Napsin A**	p504s (AMACR), CD15, p16, Vimentin	ER, AFP, CEA, sox-2	PgR, WT-1, mammaglobin, CK20, CD10, p53 (wild type)
Undifferentiated/ dedifferentiated carcinoma:	EMA, Vimentin	Pan-CK, CK8/18, p53	PAX-8, Synaptophysin, chromogranin	ER, PgR, E cadherin
Mesonephric adenocarcinoma/ mesonephric-like adenocarcinoma:	Pan-CK, CK7, EMA, PAX-8, GATA-3	TTF-1, CD10, Calretinin	ER, Napsin A	PgR, p53, PAX-2, HNF1-β, WT-1
D. Mesenchymal tumors of the uterine corpus				
Low-grade endometrial stromal sarcoma:	**CD10**[m], **CD225** (IFITM1), β-catenin, Vimentin Proliferation index (Ki-67): ~5%	ERα, PgR, bcl-2, WT-1, TLE-1	Cyclin D1, androgen receptors, actin, Desmin, CD34, pan-CK	**h- Caldesmon**, **Calponin**, EMA, inhibin, oxytocin receptor
High-grade endometrial stromal sarcoma: (YWHAE-NUTM2A/B associated)	**Cyclin D1**, BCOR	CD56, CD117	CD117	ER, PgR, CD10, DOG1
High-grade endometrial stromal sarcoma: (ZC3H7B-BCOR associated)	**Cyclin D1**, CD10	BCOR	ER, PgR, actin, pan-TRK	Desmin, DOG-1

Uterine leiomyoma/ leiomyosarcoma:	Desmin, **actin**, **Smoothelin**, **Calponin**, **h-Caldesmon**, oxytocin receptor, p16[h], p53[g], Vimentin Proliferation index (Ki-67) in: – Uterine leiomyoma: <5% – Atypical uterine smooth muscle tumors: 5–10% – Uterine leiomyosarcoma: >15%	**PgR**, WT-1, CD56	Pan-CK, ER, CD10	EMA
Undifferentiated uterine sarcoma:		p16, p53	CD10, ER, PgR	
Perivascular epithelioid tumor of the uterus:	**HMB45**, **Melan A**, Tyrosinase, MITF[h], CD63 (NK1-C3)		Actin, Desmin, CD117	CD10, CD34, DOG-1, pan-CK, S100
E. Gestational trophoblastic disease				
Exaggerated placental site:	Human leukocyte antigen G (HLA-G), human placental lactogen (hPL), GATA-3 Proliferation index (Ki-67): < 10%			P63
Placental site nodule and plaque/atypical placental site nodule:	Pan-CK, HLA-G, **PLAP**, **GATA-3**, inhibin, **p63** CD10 Proliferation index (Ki-67): 5–15%[i]	p16		hPL
Epithelioid trophoblastic tumor:	Pan-CK, **PLAP**, **GATA-3**, **p63**, inhibin Proliferation index (Ki-67): >10%			hPL, HLA-G, p16
Placental site trophoblastic tumor:	Pan-CK, HLA-G, hPL, CD146, MUC-4 Proliferation index (Ki-67): >10–30%	Inhibin	βhCG	p63
Gestational choriocarcinoma:	See choriocarcinoma of the ovary (Sect. 16.2)			
F. Tumors of the fallopian tube				
Serous tubal intraepithelial carcinoma (STIC)[j]:	**p53**, p16, Stathmin-1[k] Ki-67 > 15%			
Serous carcinoma:	CK7, CK8, CK18, CK19, EMA, WT-1, **p53**, p16	ER, PgR		CK5/6, CK20

Endometrioid adenocarcinoma:	**CK7**, CK8/18, CK19, **PAX-8**, EMA, **ER**	PgR, GFAP, Vimentin	CD56	WT-1, p53, p16, CK20, CK5/6, CEA, CDX-2
Undifferentiated carcinoma:	EMA, Vimentin	Pan-cytokeratin, CK8/18	Synaptophysin, chromogranin	ER, PgR
Adenomatoid tumor:	Pan-CK, CK5/6, CK7, **WT-1**,[o] **Calretinin**, **BAP1**, Mesothelin, Podoplanin (D2–40)[p]	Thrombomodulin (CD141)		EPCAM, CK20, p63, CEA, CDX-2, TTF-1, PAX-8, Smoothelin
G. Tumors of uterine ligaments				
Wolffian tumor:	Pan-CK, CK7, androgen receptors, Vimentin	Calretinin, CD10, Melan A	Inhibin	EMA, GATA-3, CK5/6, CK20, PAX-8, SF-1
Epithelial tumors of Müllerian type:	See uterine equivalents			
Leiomyoma/leiomyosarcoma:	See uterine equivalents			

[a] CK7 is positive in glandular components
[b] CK5/6/14 is positive in squamous components
[c] Well-differentiated neuroendocrine tumor (carcinoid)
[d] Well-differentiated neuroendocrine carcinoma (atypical carcinoid)
[e] Poorly differentiated neuroendocrine carcinoma
[f] CDX-2 may be positive in mucinous-type endometrioid adenocarcinoma
[g] p16 and p53 are markers for leiomyosarcoma, negative in benign tumors
[h] Microphthalmia transcription factor
[i] Proliferation index (Ki-67) in placental site nodule and exaggerated placental site <5%; >5% in atypical placental site nodule; 10-30% in epithelioid trophoblastic tumor and placental site trophoblastic tumor and > 70% in choriocarcinoma
[j] See Fig. 11.2
[k] Diffuse expression in STIC lesions, but only a few scattered cells in normal fallopian mucosa are present [2]
[l] PAX-2 is usually expressed in benign proliferating endocervical glands
[m] See Fig 11.4
[n] CDX-2 and PDX-1 positive in intestinal- and signet ring cell adenocarcinoma
[o] See Fig 11.5
[p] See Fig 11.6

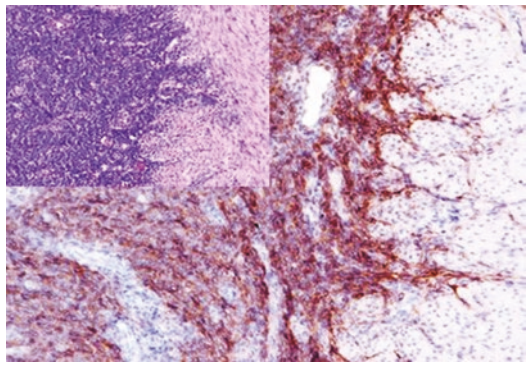


Fig. 11.4 Low-grade endometrial stromal sarcoma. Tumor cells with strong CD10 expression

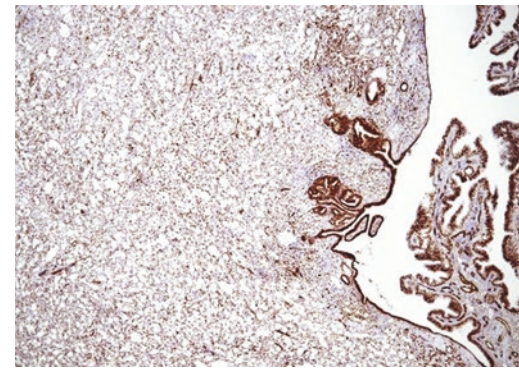


Fig. 11.5 Adenomatoid tumor of the fallopian tube with nuclear WT-1 expression in the tumor cells and the epithelial cells of the tubal mucosa

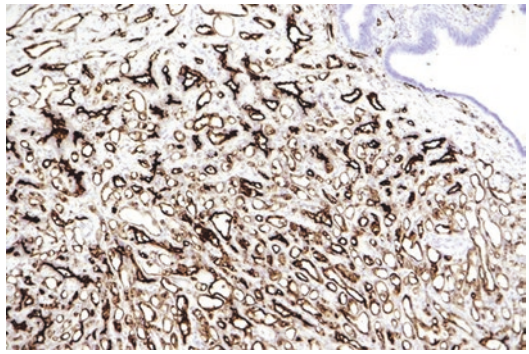


Fig. 11.6 Adenomatoid tumor of the fallopian tube with D2–40 expression in the tumor cells, whereas epithelial cells of tubal mucosa negative for D2 40

11.6 Tumors of the Ovary

11.6.1 Diagnostic Antibody Panel for Ovarian Epithelial Tumors

Cytokeratin profile, CEA, CA125, PAX-8, WT-1, Sox-17, p53, p16, GATA-3, S100P, steroid hormone receptors, and HNF-1β.

11.6.2 Diagnostic Antibody Panel for Ovarian Germ Cell Tumors

Oct-4, SALL-4, CD117, PLAP, GATA-3, Sox-2, Sox-17, AFP, CD30, βhcG, and cytokeratin profile (see also markers of testicular germ cell tumors, Sect. 13.2).

11.6.3 Diagnostic Antibody Panel for Ovarian Sex Cord-Stromal Tumors

Inhibin, anti-Müllerian hormone, Adrenal 4 binding protein (SF-1), FOXL-2, Melan A, CD56, and CD99 (see testicular sex cord-stromal tumors).

11.7 Therapy-Related Markers

Steroid hormone receptors (ER, PgR); Mismatch repair proteins (MLH1, PMS2, MSH2, MSH3, MSH6); PD-L1; p53; HER-2; folate receptor alfa (FRα); L1CAM (CD171); and Ki-67.

11.7.1 Wilms Tumor Protein-1

Wilms tumor protein-1 (WT-1)		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Nephroblastoma – Mesothelioma – Malignant melanoma – Metanephric adenoma – Ovarian serous carcinoma – Carcinoma of the fallopian tube – Mucinous breast carcinoma	Acute myeloid leukemia, Burkitt lymphoma and a subset of ALL, desmoplastic small round cell tumor, endometrial stromal sarcoma, uterine leiomyosarcoma, sex cord-stromal tumors (granulosa cell tumor, fibroma, fibrothecoma, Sertoli cell tumor), Brenner tumor, ovarian small cell carcinoma of hypercalcemic type, neuroblastoma, rhabdoid tumor, rhabdomyosarcoma, liposarcoma, angiosarcoma, osteosarcoma	Renal tissue (glomerular podocytes), myoepithelial cells, mesothelial cells, granulosa cells, Sertoli cells, mucosa of the fallopian tube, endometrial stroma, spleen, breast tissue, bone marrow stem cells
Positive control: Appendix		

Diagnostic Approach Wilms tumor protein-1 (WT-1) is a transcriptional regulator encoded by the Wilms tumor gene 1 on chromosome 11p13 with 4 isoforms. WT-1 plays an important role in the regulation of growth factors and the development of tissues from the inner layer of the intermediate mesoderm, including the genitourinary system, mesothelial cells, and spleen. Mutation within the WT-1 gene affecting the DNA-binding domain can cause the development of nephroblastoma. In routine immunohistochemistry, WT-1 shows two different expression patterns: first, a true nuclear expression pattern characteristic for different tumors such as serous carcinomas of ovarian, tubal, and peritoneal origin and mesothelioma (Fig. 11.7). Second, a cytoplasmic staining pattern found in endothelium and vascular tumors in addition to some carcinoma types such as pulmonary adenocarcinoma [18]. The cytoplasmic expression pattern appears to result from cross-reactivity with other epitopes unrelated to the WT-1 transcription factor. Endometrioid, clear cell, transitional, and mucinous carcinomas are usually WT-1 negative or show weak focal positivity. WT-1 immunohistochemistry helps differentiate between WT-1 positive tumors and many other WT-1 negative tumors with similar morphology, such as neuroblastoma and the PNET tumor group.

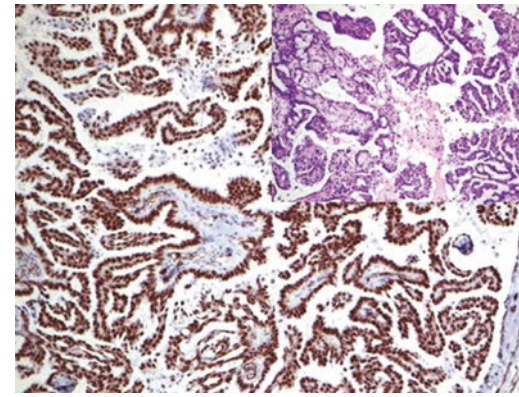


Fig. 11.7 Serous ovarian carcinoma with strong nuclear WT-1 expression

WT-1 is also a helpful marker to discriminate between malignant melanoma cells (WT-1 +) and benign nevus cells (WT-1 -) and between neoplastic endothelial cells (hemangioma) (WT-1 +) and reactive endothelial cells or vascular malformation (WT-1 -) (see Chaps. 19, 22 and 25).

Diagnostic Pitfalls WT-1 labels a high percentage of epithelioid mesotheliomas, which are to be considered in the differential diagnosis between ovarian peritoneal carcinosis and primary peritoneal mesotheliomas. Other antibodies such as PAX-8, Ber-Ep4, and Calretinin are helpful for differential diagnosis.

11.7.2 Carbohydrate Antigen 125

CA125 (MUC-16)		
Expression pattern: Membranous (luminal surface)		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Ovarian carcinoma (serous, endometrioid and clear cell carcinomas) – Endometrium carcinoma – Adenocarcinoma of the uterine cervix – Pancreatic adenocarcinoma	Lung-, breast-, gastrointestinal-, uterine-, and seminal vesicle adenocarcinomas, yolk sac tumor, epithelioid mesothelioma, anaplastic large cell lymphoma, desmoplastic small round cell tumor	Breast ductal epithelium, epithelium of lung, gastrointestinal tract, biliary system, the pancreas, female genital tract and apocrine glands, mesothelial cells
Positive control: Serous ovarian carcinoma		

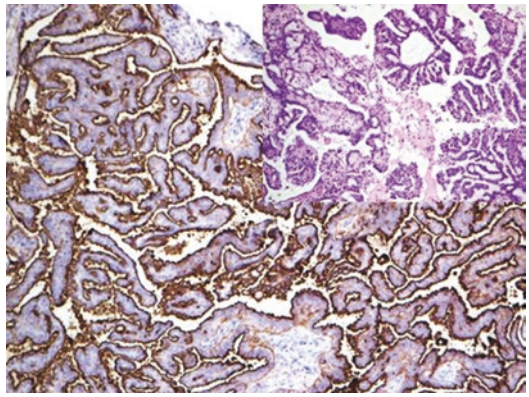


Fig. 11.8 Serous ovarian carcinoma with strong membranous CA125 expression

Diagnostic Approach Carbohydrate antigen 125 (CA125) is a high molecular weight glycoprotein classified as mucin 16 (MUC-16). CA125 is normally expressed by the glandular epithelium of different organs and is highly expressed in ovarian serous and clear cell carcinomas (Fig. 11.8). Serum CA125 is also used to monitor the progression of ovarian carcinoma.

Diagnostic Pitfalls CA125 is expressed by different epithelial and non-epithelial malignancies and lacks specificity to ovarian carcinoma. Mesotheliomas can also be positive for CA125.

11.7.3 Hepatocyte Nuclear Factor-1 β

HNF-1 β is a member of the hepatocyte nuclear factor family listed in detail in the previous chapter. HNF-1 β is used to differentiate between different types of ovarian and endometrial carcinomas.

11.7.4 PAX-8

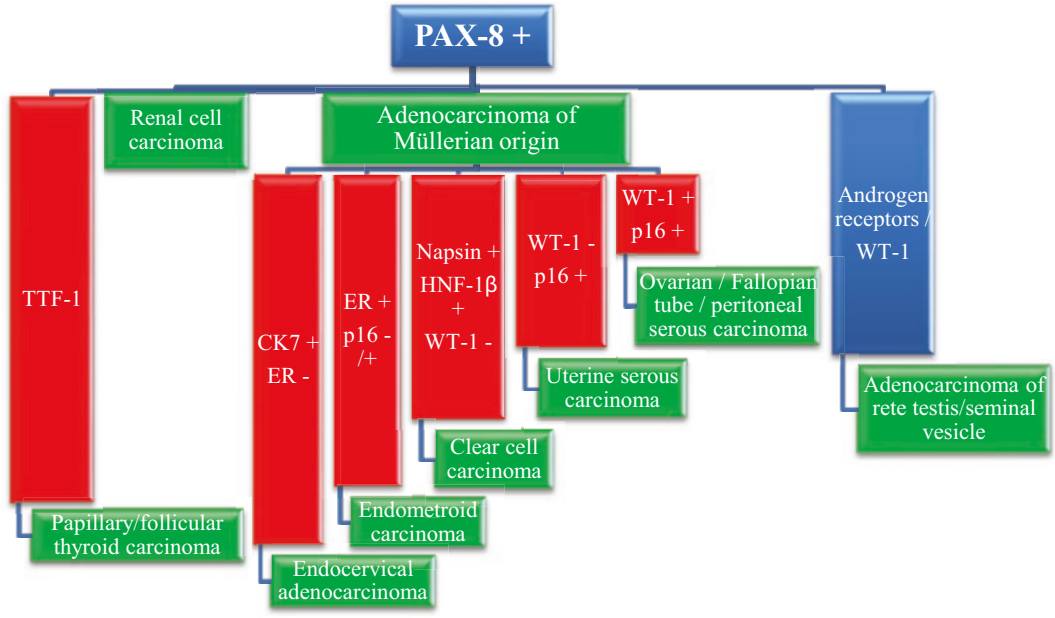
PAX-8 is a transcriptional factor and a member of the paired box (PAX) family listed in detail with the markers of renal cell tumors (Sect. 12.1). PAX-8 is highly expressed in Müllerian glandular epithelia, renal tubules, and the upper urinary system in addition to thyroid follicular cells. PAX-8 strongly labels all endocervical, uterine, and ovarian tumors of Müllerian origin, including serous, clear cell, and endometrioid carcinomas (see Algorithm 11.1). Mucinous ovarian carcinomas express PAX-8 in about 40% of the cases.

11.7.5 Sox-17

Sox-17		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
Endometrial and ovarian – Low and high-grade serous carcinoma – Endometrioid carcinomas – Clear-cell carcinomas	Yolk sac tumor, seminoma, dysgerminoma	Epithelium of the fallopian tube, endometrium and endocervical glands, endothelium
Positive control: Normal endothelium		

Diagnostic Approach Sox-17 (SRY-box transcription factor 17) is a member of the SOX family of transcription factors, involved in the regulation of embryonic development, including differentiation of endoderm, formation of vascular endothelium, and maintenance of fetal and neonatal hematopoietic stem cells. Sox-17 is normally expressed in the epithelium of the fallopian tube, endometrium, endocervical glands, and vascular endothelial cells.

Sox-17 is highly expressed in different ovarian and endometrium carcinomas, including serous, endometrioid, and clear cell carcinomas, in addition to germ cell tumors, including Yolk sac tumor, dysgerminoma, and seminoma but negative in ovarian mucinous carcinoma and sex cord-stromal tumors. Sox-17 is also expressed in endothelial tumors, including angiosarcoma (Figs.11.9 and 11.10) [19].



Algorithm 11.1 PAX-8 positive tumors

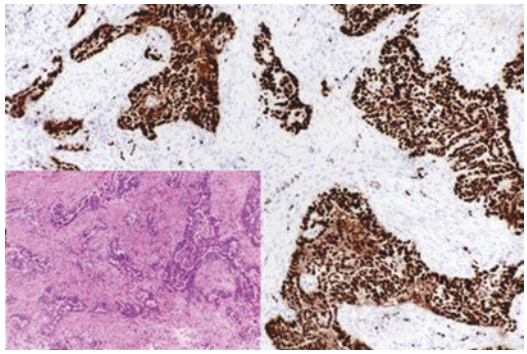


Fig. 11.9 Peritoneal biopsy infiltrated by high-grade serous ovarian carcinoma. Tumor cells exhibit strong nuclear Sox-17 expression; Sox-17 also stains the nuclei of endothelial cells

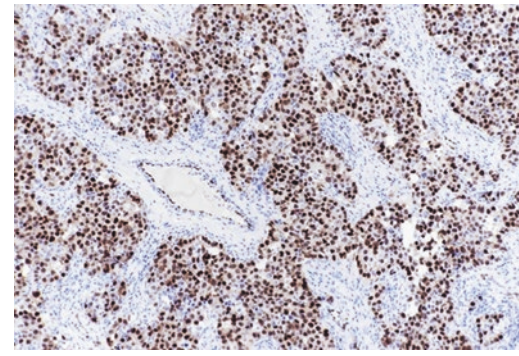


Fig. 11.10 Ovarian dysgerminoma with strong nuclear Sox-17 expression in the tumor cells. Endothelial cells also show strong nuclear Sox-17 expression

Sox-17 is not expressed in normal thyroid tissue and cells of renal tubules. Thyroid, renal, breast, bladder, colorectal, and squamous cell carcinomas are usually negative for Sox-17.

Diagnostic Pitfalls Sox-17 may be weakly positive in a subset of other carcinoma types, such as endocervical adenocarcinoma, hepatocellular carcinoma, and cholangiocarcinoma.

11.7.6 Folate Receptor

Folate receptors (FR) are a receptor family that includes four isoforms, FRα (adult), FRβ (fetal), FRγ, and FRδ, which are cell-surface glycoproteins except for FRγ, found as a secreted protein. The folate receptors are encoded on chromosome 11q13.3–14.1, bind to folic acid (vitamin B9) and its derivatives, and transport them inside the cells

essential for the biosynthesis of purines and thymidine required for DNA synthesis, methylation, and repair. In modern oncology, FRα is the therapeutic target for specific antibodies and drug-conjugated antibodies. FRα has low expression levels in limited normal tissue types with an apical membranous expression pattern. It is normally expressed in the mucosa of fallopian tubes, cells of proximal renal tubules, pneumocytes type I and II, bronchial glands, submandibular salivary glands, choroid plexus, and placental trophoblasts. FRα is overexpressed in different tumor types, including ovarian, endometrial, triple-negative breast, and lung carcinomas, in addition to mesothelioma [20, 21]. For therapeutic purposes, the expression of FRα can be detected in tumor tissue by immunohistochemistry using specific antibodies. Only membranous stained cells are considered for the interpretation of stained tumor slides, and only tumors exhibiting moderate to strong membranous expression in more than 75% of the tumor cell population are considered positive. FRβ is the therapeutic target for some acute myeloid leukemia (AML) types.

11.7.7 FOXL2

FOXL2		
Expression pattern: Nuclear		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Sex cord-stromal tumors	Squamous cell carcinoma of the cervix, breast carcinoma, pituitary gland adenoma (gonadotropin producing and null cell adenoma)	Granulosa cells, a subset of pituitary cells, eyelid
Positive control: Ovarian tissue (granulosa cells)		

Diagnostic Approach **F**orkhead b**ox** transcription factor **L2** (FOXL2) is a transcriptional factor involved in the development of the ovaries as it is essential for the maturation of ovarian follicles, maintenance of ovarian function, and normal development of the female genital tract. FOXL2 is also essential for the endocrine function of the pituitary gland.

Mutations within the FOXL2 gene associated with the strong FOXL2 expression are found in the majority of adult granulosa cell tumors; nevertheless, the expression of FOXL2 is also found in all other sex cord-stromal tumors also lacking the FOXL2 gene mutations. FOXL2 is highly expressed in testicular and ovarian sex cord-stromal tumors, including adult granulosa cell tumors, thecoma/fibroma but less common in Sertoli/Leydig cell tumors and sclerosing stromal tumors. A subset of pituitary gland adenomas is also positive for FOXL2, namely gonadotropins-producing adenomas and the majority of null cell adenomas [22–24]. Ovarian surface epithelial tumors and germ cell tumors are negative for FOXL2.

11.7.8 Adrenal 4 Binding Protein (SF-1)

This marker is listed with the markers of adrenal cortex tumors (Sect. 14.6). SF-1 is one of the best markers for sex cord-stromal tumors as it is expressed in the vast majority of adult granulosa cell tumors, Sertoli and Leydig cell tumors, and steroid cell tumors, in addition to ovarian fibroma and fibrothecoma.

Immunoprofile of ovarian tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
A. Ovarian epithelial tumors				
Serous ovarian neoplasms: – Adenoma – Borderline tumor – Low-grade carcinoma – High-grade carcinoma	CK7, CK8, CK18, CK19, EMA, CA125, **WT-1**, **PAX-8**, **p53**[a], **p16**[a], HAM56 Median proliferation index (Ki-67) in serous carcinoma: Low grade ~ 2,5% High grade ~ 22%	PAX-2, p63[b], Glut-1[b], Mesothelin	Vimentin, ER, PgR, Calretinin, S100, CK5/6, TTF-1, CD99	Villin, CK20, **CEA**, MUC-2, CDX-2, inhibin
Mucinous ovarian neoplasms (adenoma, borderline tumor and carcinoma):	CK7, CK8, CK18, CK19, EMA	CK20[c], CDX-2[c], MUC-2, MUC5AC, **CEA**, p53[d]	PAX-8, villin	**WT-1**, **p16**, **sox-17**, ER, PgR, CA125, Vimentin, inhibin, TTF-1
Endometrioid carcinoma:	CK7, CK8, CK18, CK19, EMA, **PAX-8**, **sox-17**, ER, CA125	Vimentin, Mesothelin, CD99	p16, PAX-2, CK5	CK20, **WT-1**, CEA, inhibin, TTF-1
Clear cell adenocarcinoma:	**Hepatocyte nuclear factor 1-β (HNF1-β)**, **PAX-8**, **sox-17**, CK7, EMA	**Napsin A**, Vimentin, CD15, CA125	AFP, CEA, p53, PAX-2	**WT-1**, **p16**, ER, PgR, CK20, CD10
Brenner tumor (benign/ malignant):	*Epithelial components*: EMA, **CK7**, p63, CEA, **CK5/6/14**[e], **GATA-3**, CA125, **Uroplakin III** *Fibrous stroma*: Vimentin	**WT-1**, AR, S100P, bcl-2		PAX-8, CK19, CK20, Thrombomodulin (CD141), CDX-2, p16, ER, PgR, Vimentin Pan-CK
Mesonephric-like adenocarcinoma:	CK7, PAX-8, TTF-1, GATA-3		CD10	WT-1, ER, PgR
Undifferentiated/ dedifferentiated carcinoma	Pan-CK		PAX-8	ER, PgR
B. Sex cord-stromal tumors				
Adult granulosa cell tumor:	**FOXL2**, **adrenal 4 binding protein (SF-1)**, **inhibin**, Vimentin	Calretinin, **CD99**, actin, S100, CD56, **WT-1**, Melan- A, ER-β, PgR	Pan-CK, CK8, CK18, ER-γ	CK7, EMA, CEA, anti-Müllerian hormone, PAX-8, β-catenin, Desmin
Juvenile granulosa cell tumor:	**Inhibin**, Calretinin, CD99, WT-1	EMA		β-Catenin
Thecoma/fibroma/ fibrosarcoma:	**Inhibin**, Calretinin, **FOXL2**, **adrenal 4 binding protein (SF-1)**, **WT-1**, CD56, Vimentin	Sm-actin, Calretinin	ER, PgR	Pan-CK, CD10
Sclerosing stromal tumor:	Sm-actin, PgR, **FOXL2**, Vimentin	**Inhibin**, Calretinin, Desmin	ER, TFE-3	Pan-CK, EMA
Leydig cell tumor:	**Inhibin, adrenal 4 binding protein (SF-1)**, Melan-A, Calretinin, Vimentin	CD99, CD56	Pan-CK, S100, actin, Desmin, Synaptophysin, chromogranin, EMA	PLAP, **β-catenin**, AFP, CEA, Oct-4, SALL-4
Sertoli cell tumor:	**Inhibin, adrenal 4 binding protein (SF-1)**, **β-catenin**, **anti-Müllerian hormone**, WT-1, Melan A, Vimentin	AFP, **FOXL2**, CD56, CD99, pan-CK, Calretinin, NSE, S100	Synaptophysin, chromogranin	EMA, CK7, PLAP, PAX-8, GATA-3, Oct-4, SALL-4, CEA

Microcystic stromal tumor:	WT-1, β-catenin[f], CD10, FOXL2, SF-1, cyclin D1		AR	Inhibin, Calretinin, ER, PgR, EMA
Signet-ring stromal tumor:	SF-1, Calretinin, β-catenin, CyclinD1, actin		Pan-CK	Inhibin, EMA
Steroid cell tumor:	Inhibin, Melan A, Calretinin			FOXL2
Sex cord tumor with annular tubules:	**Inhibin**, **adrenal 4 binding protein (SF-1)**, WT-1, Calretinin	CD56	Pan-CK	EMA
C. Germ cell tumors				
Dysgerminoma:	**SALL4**, **Oct-4**, **NANOG**, **PLAP**, **CD117**	Pan-CK, D2–40	CK8/18	AFP, ßhcG, sox-2, inhibin, S100, EMA
Embryonal carcinoma:	**SALL-4**, **NANOG**, **sox-2**, **PLAP**, **AFP**, **CD30**, **Oct-4**, pan-CK	CK19, NSE		ßhcG, **EMA**, CEA, CD117, Vimentin
Yolk sac tumor:	**AFP**, **SALL-4**, Pan-CK, CD10, Glypican-3	GATA-3, PLAP, CDX-2	HepPar1	**EMA**, CD30, ßhcG, Oct-4, **sox-2**, CK7, Vimentin
Choriocarcinoma:	*Syncytiotrophoblastic cells*: **ßhcG**, inhibin, **GATA-3**, CD10, SALL-4, pan-CK, CK8/18, CK19, p63, EGFR	**PLAP**, human placental lactogen, EMA, CEA	Vimentin	CD30, AFP, Oct-4
	Cytotrophoblastic cells: CD10, pan-CK, CK8/18, CK19, CEA	PLAP		ßhcG, inhibin, EMA, CD30, AFP, Oct-4
Polyembryoma:	*In embryonal bodies*: **AFP**, pan-CK	PLAP		
Gonadoblastoma:	*Germ cells*: PLAP, CD117, Oct-4, NANOG, D2–40 *Sex cord cells*: Inhibin, WT-1, Vimentin	Pan-CK		
D. Mesenchymal tumors				
Endometrioid stromal sarcoma:	CD10, ER, PgR	WT-1		
Ovarian myxoma:	Vimentin	Actin		Pan-CK, Desmin, S100
E. Miscellaneous tumors				
Female adnexal tumor of probable Wolffian origin (ovarian Wolffian tumor):	Pan-CK, CK7, **androgen receptors**, Vimentin	Calretinin, CD10, Melan A	Inhibin	EMA, GATA-3, CK5/6, CK20, PAX-8, SF-1
Solid pseudopapillary tumor:	CD10, CD56, CD99, WT-1, β-catenin	PgR,	CD117	Inhibin, Calretinin
Small cell carcinoma, hypercalcemic type:	EMA, **WT-1**, p16, p53	Calretinin, CD56, SALL-4	Synaptophysin, chromogranin	CD10, inhibin, TTF-1
Small cell carcinoma, pulmonary type:	NSE, **CD56**	Pan-CK, TTF-1	Synaptophysin, chromogranin	

[a] High expression levels of p16 and p53 are characteristic for high-grade serous carcinoma and low expression levels or negativity characteristic for low-grade carcinoma. p53 is constantly negative in the case of nonsense-type mutations
[b] Stain intensity correlates with the grade of malignancy [25]
[c] CDX-2 and CK20 are positive in mucinous adenocarcinoma and intestinal-type adenoma
[d] Usually negative in adenoma and borderline tumors
[e] CK5/6/14 positive in basal epithelial cells
[f] Nuclear and cytoplasmic

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