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# Molecular and Immunohistochemical Markers of Diagnostic and Prognostic Value in Testicular Tumors

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## Introduction

Primary testicular neoplasms are rare with less than 10,000 new cases reported each year in the USA. Once a testicular mass is resected, it is imperative to establish an accurate diagnosis since the most common tumors are mostly curable as long as the therapeutic approach is directed to the specific tumor type. Testicular germ cell tumors (GCTs) have a very good prognosis, even in the presence of metastatic disease, due to their exquisite sensitivity to cisplatin-based chemotherapy. However, the therapeutic approach to clinical stage I primary testicular GCTs will vary significantly based on the pathological diagnosis rendered. In cases of pure seminoma, following orchiectomy, the patients are either placed on surveillance or given low-dose radiation therapy. No pathological features in the primary tumor, including lymphovascular invasion and rete testis involvement, have been validated to predict progression or are utilized to drive subsequent therapy. In mixed GCTs, lymphovascular invasion and the percentage of embryonal carcinoma (EC) have been independently associated with an increased risk of disease progression in multiple studies. For this reason in many institutions, particularly in Europe, these patients will be given a modified protocol of systemic chemotherapy

while, in others, a primary retroperitoneal lymph node dissection will be the treatment of choice. Sex cord-stromal tumors are primarily a surgical disease since no systemic therapies have shown significant efficacy. The hallmarks to cure include early detection and surgical removal of all existing disease. Prognostic factors are morphology based since no molecular markers have been identified to predict prognosis or response to therapy.

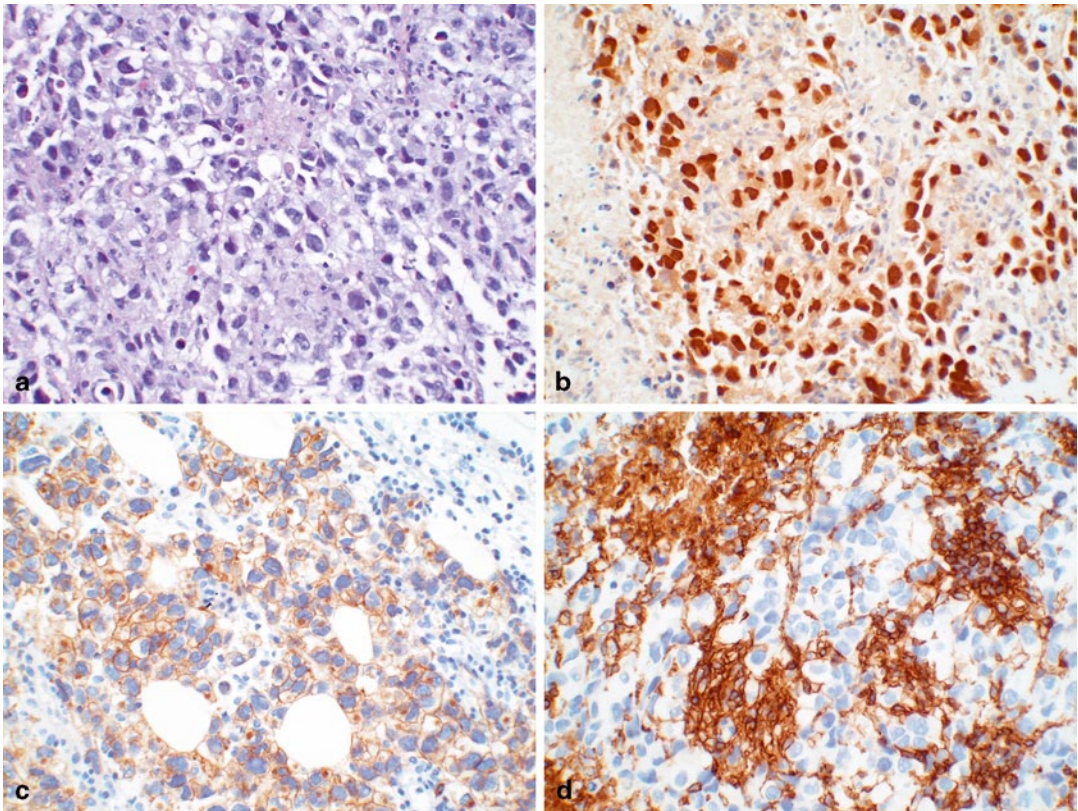
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## Germ Cell Tumors

Given the relative rarity of testicular GCTs and their vast morphological heterogeneity, it is understandable that pathologists in routine clinical practice resort to ancillary studies, particularly immunohistochemistry, to establish the correct diagnosis. Another very important clinical scenario where the pathologic diagnosis is critically important is when evaluating retroperitoneal and mediastinal masses (midline masses of unknown origin), particularly in younger patients. While these masses may very well represent sarcoma or lymphoma, for example, GCTs should always enter in the differential diagnosis (Fig. 40.1a–d). Fortunately, there are multiple assays that can help us to establish the histogenesis of the lesion. Unfortunately, the rarity of the disease, the complex morphologies that can be present as well as the abundance of markers that have been published through the years as helpful in distinguishing between various entities have led to overdependence and overuse of ancillary studies

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**Fig. 40.1** Seminoma in a needle biopsy of the retroperitoneum. **a** H&E stain. **b** OCT3/4 showing nuclear immunoreactivity. **c** CD117 showing cytoplasmic/membranous staining. **d** CD20 staining surrounding lymphocytes

with its predictable negative impact on healthcare costs. The purpose of this chapter is to review the assays that are most useful in arriving at the correct diagnosis, incorporating novel markers that have entered the clinical laboratory in recent years, and limiting/eliminating the use of markers that are either of limited utility or have been supplanted by newer assays with better performance characteristics. As is the case in virtually all tumors, there is no single marker that is 100% specific or sensitive for any given tumor type. For this reason, markers should always be used in a panel, but this panel should be directed to the specific differential diagnosis under consideration.

### Molecular Markers of Germ Cell Lineage

Through the years, a modest but significant amount of work has gone into understanding the

molecular pathogenesis of testicular GCTs, the genomic differences among various tumor types, and the genes that are differentially expressed in chemotherapy-sensitive as well as chemotherapy-resistant tumors [1–3].

However, none of these findings are used clinically, either to establish a precise diagnosis (subclassification) or to choose therapy. At this time, the key to selecting proper therapy lies on an accurate pathological diagnosis, assessment of pathologic risk factors, state-of-the-art imaging, and serum tumor markers.

Testicular GCTs can be divided into three groups (infantile/prepubertal, adolescent/young adult, and spermatocytic seminoma (SS)), each with its own constellation of clinical history, molecular, and clinical feature [4–7]. They originate from germ cells at different stages of development. Tumors arising in prepubertal gonads are either teratomas or yolk sac tumors (YSTs),

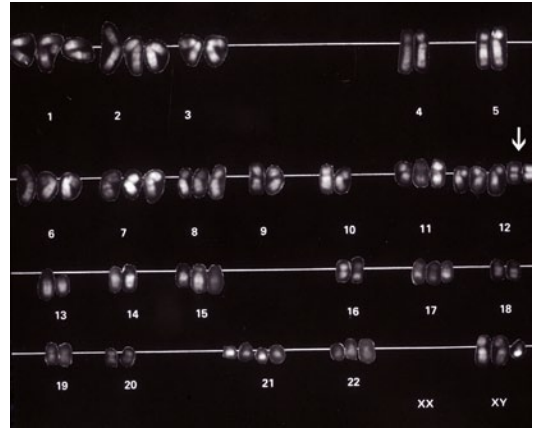
**Table 40.1** Molecular characterization of testicular germ cell tumors (GCTs)

i(12p) is the most common genetic abnormality seen in testicular germ cell tumors
Presence of i(12p) is characteristic but not entirely specific for germ cell tumor origin
Presence of i(12p) is of diagnostic utility but does not predict histologic type, prognosis or response to therapy
i(12p) is best identified by karyotype analysis
Presence of i(12p) may be analyzed by fluorescent in situ hybridization (FISH) in formalin-fixed, paraffin-embedded tissues, but this assay suffers from less than ideal sensitivity and specificity

tend to be diploid, and are not associated with i(12p) nor intratubular germ cell neoplasia unclassified (IGCNU). The annual incidence is approximately 0.12 per 100,000. SS arises in older patients. These benign tumors may be either diploid or aneuploid and have losses of chromosome 9 rather than i(12p). Intratubular SS is commonly encountered but IGCNU is not. Their annual incidence is approximately 0.2 per 100,000. The pathogenesis of prepubertal GCTs and SS is poorly understood.

The most common testicular GCTs arise in postpubertal men and are characterized genetically by the presence of excess genetic material of the short arm of chromosome 12, usually due to one or more copies of i(12p), or other forms of 12p amplification and aneuploidy [8] (Table 40.1). The consistent gain of genetic material from the short arm of chromosome 12 seen in these tumors suggests that it has a crucial role in their development. IGCNU is the precursor to these invasive tumors.

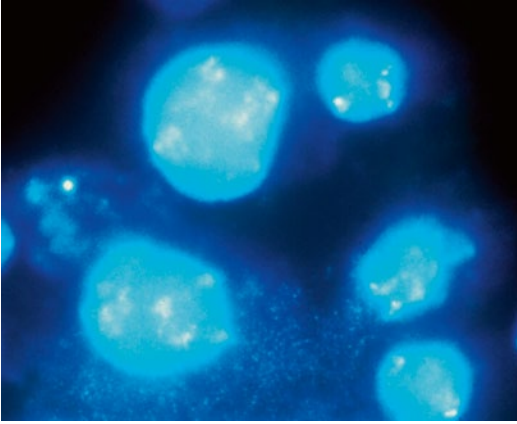
While IGCNU is considered to be the precursor of all GCTs, the stage in germ cells development at which transformation occurs is not known. One model proposed by Skakkebaek and colleagues suggests that fetal gonocytes (primordial germ cells) undergo abnormal cell division (polyploidization) *in utero*, primarily due to environmental factors. These cells undergo abnormal cell division mediated by a kit receptor/kit ligand (stem cell factor) paracrine loop, leading to uncontrolled proliferation of gonocytes. Subsequent invasive growth may be mediated by postnatal



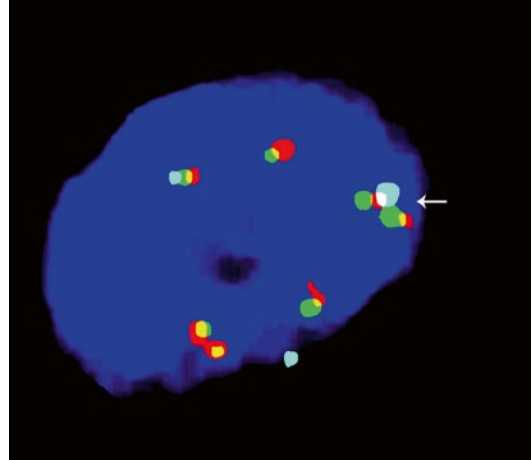
**Fig. 40.2** An early karyotype depicting the presence of two copies of isochromosome 12p [i(12p)] (arrow). It was these early studies that allowed investigators to define what we now regard as the most common genetic abnormality seen in adult onset testicular germ cell tumors (GCTs)

and pubertal gonadotrophin stimulation. In this model, i(12p) is seen only after stromal invasion occurs [9]. A second model proposed by Chaganti and colleagues suggests that aberrant chromatid exchange events during meiotic crossing-over may lead to increased 12p copy number and overexpression of cyclin D2 (*CCND2*). In a cell containing unrepaired DNA breaks (recombination associated), overexpressed cyclin D2 may block a p53-dependent apoptotic response and lead to reinitiation of cell cycle and genomic instability. This aberrant, genomically unstable cell is now able to escape the apoptotic effects of p53 and may re-enter the cell cycle as a neoplastic cell. In this model, i(12p) is present in IGCNU [10].

Given the fact that i(12p) or at least excess genetic material of 12p is characteristic of postpubertal GCTs of the testis, it should not come as a surprise that its presence can and has been used as a diagnostic assay. This genetic abnormality is not absolutely pathognomonic of germ cell neoplasia, yet it is a very useful diagnostic tool in selected circumstances due to its rare occurrence in other solid tumors [11–13]. Classically, i(12p) is a feature best seen by karyotype which by definition requires a metaphase spread (Fig. 40.2). However, in daily practice, this assay is rarely



**Fig. 40.3** Single-color FISH assay exhibiting large, irregular signals that may represent several copies of *i*(12p) (*left*). Normal signal can be seen in an adjacent cell (*right*)



**Fig. 40.4** Three-color FISH probe exhibiting *i*(12p) (*arrow*). The aqua centromeric probe is flanked by two red-green probes

performed in solid tumors, given the popularity of modern molecular techniques. Because the possibility of germ cell origin is usually determined after the H&E slides are reviewed, many investigators have used fluorescent in situ hybridization (FISH) to establish the presence of *i*(12p) in a formalin-fixed, paraffin-embedded (FFPE) tumor-bearing tissue. In fact, multiple papers have been written documenting its utility [14–17] (Figs. 40.3 and 40.4). The most common assay is a library probe covering 12p, sometimes combined with a chromosome 12 centromeric probe. In some cases, a three-color probe is used that includes a portion of 12q. In theory, this approach is more scientifically sound since the goal is to identify excess genetic material of 12p rather than simply chromosomal gains commonly seen in any form of genomically unstable tumors. What all these studies have failed to do is to establish the sensitivity and specificity of their assay. As you can imagine, an assay that depends on the identification of abnormally large, irregular signals in a resting cell can be difficult to evaluate and is prone to problems in interpretation (Figs. 40.3 and 40.4). Now that other molecular assays that can utilize FFPE tissues have entered the diagnostic arena, it is imperative that we develop a more precise assay. One possibility that is being explored is using a single-nucleotide polymorphism (SNP) array,

which has the advantage of providing very good copy number data as well chromosome gains and losses. While the price of this assay at the present time is high, the clinical penalty that is paid if this diagnosis is missed certainly merits the financial cost.

### Immunohistochemical Markers

Immunohistochemical markers can be a very powerful ancillary tool in classifying GSTs [18]. With experience, the overwhelming majority of tumors can be classified on high-quality H&E slides alone. However, the relative low incidence of these tumors is such that any given pathologist is likely to encounter no more than a handful of cases a year. This fact combined with the wide morphologic spectrum that can be seen in these tumors makes it very likely that the pathologist will be overly dependent on ancillary studies to arrive at a final diagnosis. It is best to first elaborate a differential diagnosis based on morphology and then decide what panel of antibodies should be ordered. Too often, I encounter cases in which virtually every marker associated with any type of GCT has been ordered, even though the differential diagnosis is rather limited. This “shotgun” approach should be avoided.

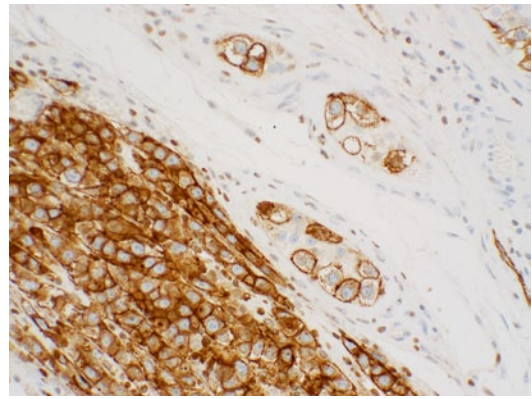


## Intratubular Germ Cell Neoplasia (IGCNU)

IGCNU can be seen adjacent to invasive GCTs in virtually all cases in which residual testicular parenchyma is present [19]. It is present in up to 4% of cryptorchid patients, up to 5% of contralateral gonads in patients with unilateral GCT, and up to 1% of patients biopsied for oligospermic infertility. Its association with testicular GCTs arising in prepubertal patients is still a source of controversy [4, 20, 21]. If present, it certainly does not have the same morphology or immunophenotype than what is seen in postpubertal gonads. Clinically, there are two settings in which it may be critical to confirm the presence of IGCNU: in the evaluation of a testicular biopsy at the time of infertility work up and when evaluating a testicular mass that is difficult to classify.

IGCNU cells contain glycogen and thus are PAS positive, diastase sensitive. Rarely will other intratubular cells, whether spermatogonia, spermatocytes, or Sertoli cells, show similar positivity. Placental-like alkaline phosphatase (PLAP) is one of the isoforms of alkaline phosphatase. PLAP antibodies will stain IGCNU, the majority of seminomas and ECs as well as a smaller percentage of YSTs. Immunoreactivity is seen in virtually all cases of IGCNU, and the staining pattern is usually membranous or cytoplasmic. No other non-neoplastic intratubular cells are immunoreactive for PLAP, but immunoreactivity may be seen in other types of nongerm cell malignancies [22, 23]. Because better markers have been developed over the years, we hardly ever use PLAP in our workup of testicular tumors. C-kit (CD117), a tyrosine kinase receptor expressed on stem cells, is overexpressed in a large percentage of IGCNU as well as seminomas, but not in other GCTs [24]. The staining pattern is cytoplasmic/membranous (Fig. 40.1c). Despite the overexpression of this antigen, CD117 is rarely mutated in these tumors. Care must be taken when interpreting CD117 in IGCNU since spermatogonia may occasionally express this antigen.

Other antibodies which immunoreact with IGCNU but are rarely used in clinical practice include M2A and 43-F [22, 25, 26]. POU5F1



**Fig. 40.5** Podoplanin (D2-40) showing strong cytoplasmic/membranous staining in intratubular germ cell neoplasia (*right*) and seminoma (*left*). This stain may be used interchangeably with CD117, but there is no need to perform both

(OCT3/4) is a very interesting marker with great clinical utility [27]. The gene serves as a transcription factor, and its product is expressed in pluripotent mouse and human embryonic stem cells and is down-regulated during differentiation. Since the gene is also required for self-renewal of embryonic stem cells, knocking out the gene is lethal. This antigen is expressed solely in IGCNU, seminoma, and EC, suggesting that these are the types of GCT cells with pluripotency, i.e., with capacity to differentiate. As a transcription factor, staining is localized to the nucleus (Fig. 40.1b).

Another transcription factor expressed in IGCNU is SALL4; however, this nuclear marker is expressed in a wider spectrum of GCTs including seminoma, EC, YST, and some glandular elements of teratoma [28]. As such, it is a useful marker in the characterization of GCTs but cannot be used in isolation. Podoplanin (clone D2-40), a transmembrane mucoprotein expressed on fetal germ cells, lymphatic endothelium, and mesotheliums, is an excellent cytoplasmic (membranous) marker with staining restricted to IGCNU and seminoma (Fig. 40.5) [29]. Since the expression patterns for CD117 and D2-40 overlap, there is no need to perform both assays.

There are several situations in which it is critically important to establish the presence or

absence of IGCNU and in which immunohistochemistry may be of great utility. These include during the workup of infertility and in the presence of a poorly differentiated testicular neoplasm in order to establish germ cell lineage (Fig. 40.1a–d). However, there is absolutely no reason to perform tests for all of the markers mentioned above. While one antibody may be sufficient, I usually test for two antigens, OCT4 and either CD117 or D2-40. PAS, PLAP, M2A, and 43-F are of no additional clinical utility and I discourage their use. In this situation, SALL-4 adds no additional information to OCT4.

## Seminoma

Seminomas are the most common GCTs arising in the male gonad, whether they arise in a pure state or mixed with other morphologic types [30–32]. “Pure” seminoma account for 30% of testicular GCTs and another 15–20% contain syncytiotrophoblasts without other germ cell components. Seminoma cells contain glycogen (PAS positive) and express PLAP, CD117, OCT3/4, and SALL-4 by immunohistochemistry but not cytokeratins, CD30, or inhibin (Table 40.2) [23, 33–37]. In fact, the immunophenotype of seminoma is virtually identical to IGCNU. In our practice, PAS and PLAP are rarely relied upon because of the availability of better discriminating markers. On occasion, weak CD30 cytoplasmic immunoreactivity may be encountered in isolated seminoma cells, a finding that should not warrant a change in diagnosis. It is important to keep in mind that CD30 may be expressed in some hematopoietic cells as

well, so attention to nuclear detail is warranted. A minority of seminoma cells may express focal and weak, dot-like, or linear immunoreactivity for cytokeratin AE1/AE3 and CAM 5.2. However, there is never diffuse and strong staining throughout the cytoplasm. Caution must be taken when interpreting cytokeratin markers since syncytiotrophoblasts are usually strongly immunoreactive, as are their mononuclear variants. If one relies on panels of markers, this issue is resolved easily since syncytiotrophoblasts lack immunoreactivity to OCT4, SALL4, D2-40, etc. Like IGCNU, seminoma cells express OCT4 in a nuclear distribution [24, 37]. SALL4 is positive in a nuclear distribution, while CD117 and D2-40 are expressed in a cytoplasmic/membranous distribution, once again similar to IGCNU (Fig. 40.5).

In practical terms, the panel of markers used to establish a diagnosis of seminoma will depend on the differential diagnosis. What is important to remember is that no other invasive GCTs will exhibit diffuse immunoreactivity for CD117 and D2-40, while seminoma shares OCT4 nuclear positivity with EC and SALL4 nuclear immunoreactivity with EC and YST.

## Spermatocytic Seminoma

SSs are rare, comprising less than 2% of testicular neoplasms [30]. They represent an entirely separate and distinct clinicopathologic entity from classic seminoma. The peak incidence is in the sixth decade of life (median 54 years). Patients as old as 87 years and as young as 25 years of age have been affected. This tumor occurs only in the

**Table 40.2** Immunohistochemical marker expression in primary testicular germ cell tumors (GCTs)

Marker	IGCNU	Seminoma	Embryonal Ca	Yolk sac tumor
OCT4 <sup>a</sup>	Positive	Positive	Positive	Negative
CD117	Positive, membranous	Positive, membranous	Negative (weak/focal positivity can be seen)	Negative (weak/focal positivity can be seen)
D2-40 <sup>a</sup>	Positive	Positive	Negative	Negative
CD30 <sup>a</sup>	Negative	Negative	Positive	Negative
SALL4	Positive	Positive	Positive	Positive
Glypican 3 <sup>a</sup>	Negative	Negative	Negative	Positive
Cytokeratin <sup>b</sup>	Negative <sup>+</sup>	Negative <sup>+</sup>	Positive	Positive

<sup>a</sup> Denotes marker of choice

<sup>b</sup> Either CAM 5.2 or AE1/AE3

male gonad, is not associated with either cryptorchidism, other types of GCTs, or i(12p). Tumor cells do not contain glycogen (negative PAS stain). Immunohistochemical stains for PLAP are negative, although occasional cells may be weakly immunoreactive. Cytokeratin stains are negative, although occasional cells may exhibit dot-like cytoplasmic staining. CD30, OCT4, and podoplanin are negative, while some investigators have reported variable immunoreactivity for CD117 (C-kit) and SALL4 [24, 30, 38].

## Embryonal Carcinoma

In its pure form, EC comprise up to 3% of GCTs, although approximately 40% of all GCTs contain an EC component. Over 50% of tumors with either pure or predominant EC components will present with metastatic disease. Pure EC most commonly occurs in patients during the third or fourth decades of life, with an average age of 32 years and is extremely rare in prepubertal children [30]. Up to 10% of patients present with symptoms related to metastatic disease. Metastases most commonly occur first to retroperitoneal lymph nodes. Twenty percent of patients present with metastatic disease with a smaller percentage also having supradiaphragmatic involvement.

The cells of EC usually show intense and diffuse immunoreactivity for PLAP, OCT4, SALL4, as well as cytokeratins (AE1/AE3 and Cam 5.2) (Fig. 40.6a–d). CD30 (Ber-H2) is a member of the tumor necrosis factor receptor family and has a cytoplasmic membrane and Golgi distribution. It is expressed exclusively in EC (Fig. 40.7). SOX-2 is a transcription factor involved in embryonic development and specifically plays a role in maintenance of pluripotency in undifferentiated embryonic stem cells [37, 39, 40]. It is expressed in a nuclear distribution in the majority of ECs but not seminoma (only isolated cases) or YSTs. While it may serve as an alternative to CD30, the assay is difficult to standardize in a CLIA complicate automated environment so we do not use it clinically. Only about 2% of ECs react with epithelial membrane antigen (EMA). Staining for CD117 protein generally is negative,

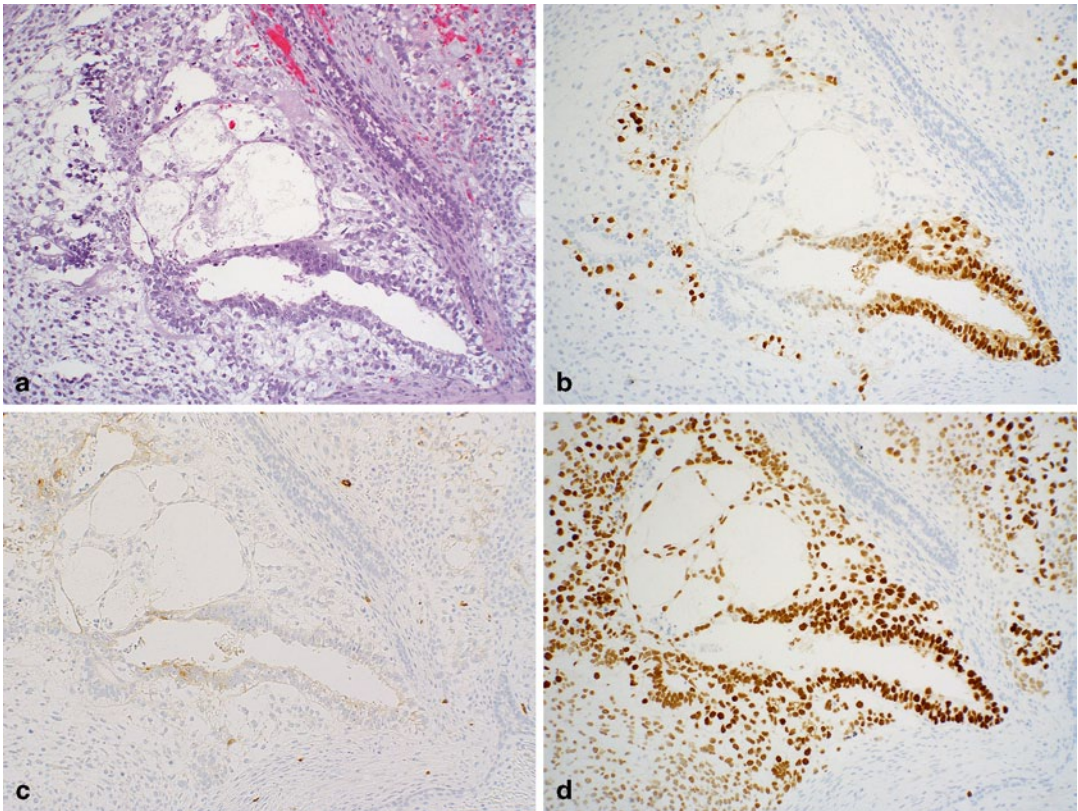
although some cells may exhibit low level of staining.  $\beta$ -HCG is demonstrable only in intermingled syncytiotrophoblastic cells [24]. Most AFP-positive foci in ECs probably represent unrecognized foci of YST or early transition to YST. Pertinent negatives include CD117 and D2-40, remembering that the former can rarely stain isolated cells.

## Yolk Sac Tumor

YSTs are characterized by multiple patterns of growth that recapitulate the yolk sac, allantois, and extra embryonic mesenchyme. It has a bimodal age distribution: infants and young children and postpubertal males. In children, it commonly presents in its pure form, usually within the first 2 years of life. YSTs account for 75% of childhood testicular GCTs. In the postpubertal setting, YST rarely presents in a pure form but is present in almost half of mixed GCTs [32, 41]. The incidence of a YST component is higher in primary mediastinal GCTs.

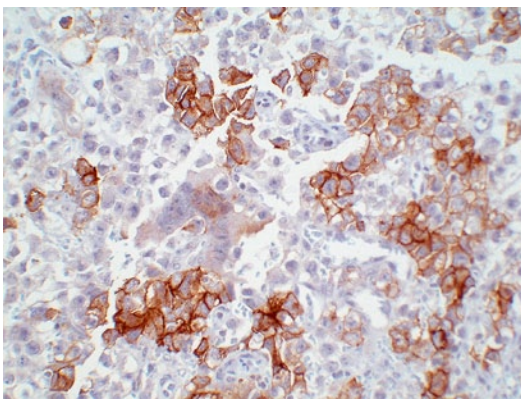
Tumor cells of YST are usually immunoreactive for low molecular weight cytokeratins and  $\alpha$ -fetoprotein (AFP), a plasma protein produced by the yolk sac and liver in the fetus. We have found that AFP immunohistochemistry can be difficult to interpret. Staining can be weak, patchy, and it is often associated with a dirty background. While we admit that some of these findings could be laboratory associated, the fact of the matter is that we never perform or interpret this assay in isolation. Because we now have better antibodies, we do not consider this assay to be absolutely necessary. PLAP staining is variable and may be absent. As previously stated, we rarely perform this assay since it lacks discriminatory power. CD117, CD30, and OCT3/4 are usually negative, but SOX2, SALL4, and Glypican 3 are positive [24, 39, 42, 43]. Glypican 3 is a membrane-anchored heparin sulfate which expressed in virtually all YSTs and in the majority of choriocarcinomas (Fig. 40.8). Expression can be seen in the epithelial component of some teratomatous glands and some trophoblastic elements, but it is otherwise limited to YSTs.



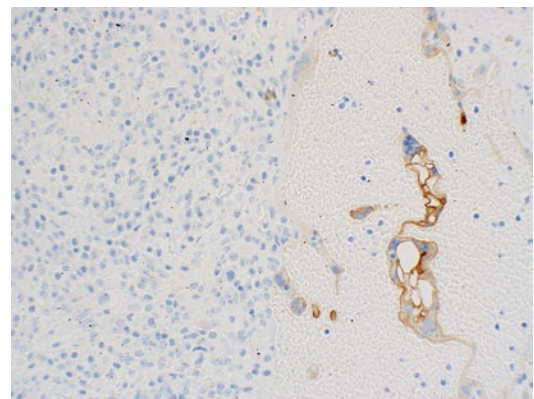


**Fig. 40.6** Mixed germ cell tumor (GCT) composed of embryonal carcinoma (EC), yolk sac tumor (YST), and teratoma. **a** H&E stain. **b** OCT3/4 nuclear immunoreactivity is limited to EC. **c** CD117, although usually limited

to seminoma, may be weakly positive in EC, as it is here. **d** SALL4 nuclear staining is seen in EC and YST but not in the epithelial teratomatous elements



**Fig. 40.7** Cytoplasmic staining for CD30 in embryonal carcinoma (EC) while the *adjacent* seminoma cells are negative



**Fig. 40.8** Glypican 3 immunoreactivity in syncytiotrophoblasts *adjacent* to unreactive seminoma



## Choriocarcinoma

Choriocarcinoma is composed of syncytiotrophoblastic, cytotrophoblastic, and other trophoblastic cells. It comprises less than 1% of testicular GCTs in its pure form; however, it may be encountered as a component of a mixed GCT in up to 15% of cases [30]. In its pure form, these highly malignant tumors occur in the second and third decades of life, are commonly associated with very high levels of serum HCG (usually above 50,000 mIU/mL), and exhibit metastatic disease at the time of initial presentation. In this setting, metastatic disease is found in not only the usual sites for other testicular tumors, but also via hematogenous spread to viscera, including lungs, liver, gastrointestinal tract, spleen, brain, and adrenal glands.

Syncytiotrophoblasts are immunoreactive with  $\beta$ -hCG as well as inhibin, epithelial EMA, low molecular weight cytokeratins, and Glypican 3. Human chorionic gonadotrophic (hCG) is a dimeric glycoprotein that is produced by placental trophoblastic cells, predominantly the syncytiotrophoblasts. The beta-chain is unique to hCG and thus is the best antigenic marker for it. Essentially all choriocarcinomas are positive for  $\beta$ -hCG, but such reactivity is often limited to syncytiotrophoblasts or intermediate trophoblasts. Cytotrophoblasts are either negative or weakly positive for  $\beta$ -HCG. PLAP may be positive but staining is variable [30]. Pregnancy-specific  $\beta$ 1-glycoprotein and human placental lactogen also are positive in syncytiotrophoblasts and intermediate-sized trophoblasts but are negative in cytotrophoblasts [32, 44].

## Teratoma

The term teratoma refers to neoplasms composed of tissues, which have differentiated along any of the three somatic pathways: ectoderm, mesoderm, or endoderm [30, 32, 45]. Tumors composed of only one of these components are regarded as monodermal teratomas. Teratomas may be composed of mature tissues, embryonal-type tissues, or a mixture of both. Historically,

they were subclassified as immature and mature forms based on their degree of differentiation. The World Health Organization (WHO) now recommends that these morphologies be considered as a single entity based on their overlapping genetic and clinical features [30].

The immunohistochemical profile of teratomas will depend on the histologic component present. In addition, glandular elements are likely not to be immunoreactive for SALL4 and Glypican 3, although focal staining may be evident for either marker; whether this represents early yolk sac differentiation is unknown. AFP may also be focally positive in glandular elements, which may be associated with the elevation of AFP in the cystic fluid [46].

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## Sex Cord-Stromal Tumors

The term refers to neoplasms containing Leydig (interstitial) cells, Sertoli cells, granulosa cells, or theca cells. While tumors may be made up of one or a combination of these cell types in varying degrees of differentiation, mixed histologic types are common in the ovary but rarely occur in the male gonad. The terminology used to describe these tumors is confusing and controversial, but it is best to adhere to the classification set forth by the WHO [30]. Sex cord-stromal tumors comprise 4–5% of primary testicular neoplasms. The morphology of these lesions is less complex than in GCTs, yet they may be difficult to classify and may be confused with other neoplasms, including tumors of germ cell origin, particularly YST and seminoma, or with mesothelioma, adnexal tumors as well as metastatic disease. There is very little known about the molecular underpinnings of this disease, except that some tumors may be associated with syndromes such as Peutz–Jeghers and Carney's syndromes. Approximately 10% of tumors show malignant behavior. Surgery remains the mainstay of treatment since no form of systemic therapy has shown significant efficacy in treating the disease. To date, there are no molecular markers that can predict clinical outcome or response to treatment.

**Table 40.3** Immunohistochemical markers most useful in identifying sex cord-stromal tumors. (Expression of any of these markers may be focal)

Marker	Leydig cell tumor	Sertoli cell tumor	LC calcifying Sertoli cell tumor	Granulosa cell tumor, adult type	Granulosa cell tumor, juvenile type
Inhibin <sup>a</sup>	Positive	Variable <sup>b</sup>	Positive	Positive	Positive
WT-1 <sup>a</sup>	Positive	Positive	Positive	Positive	Positive
Calretinin	Positive	Positive	Positive	Positive	Positive
SF-1 <sup>a</sup>	Positive	Positive	Positive	Positive	Positive
FOXL2 <sup>a</sup>	Negative	Negative	Negative	40% positive	Unknown
Cytokeratin	Negative	40% positive	Negative	Usually negative	Positive only in cells lining microcysts

<sup>a</sup> Denotes marker of choice, most likely to be of diagnostic utility

<sup>b</sup> Inhibin positivity is less commonly seen in SCTs as compared to LCTs

### Leydig Cell Tumor (LCT)

LCTs are the most common pure testicular sex cord-stromal neoplasms and account for 1–3% of testicular neoplasms [30, 32, 47]. They may occur at any age, though most common between the third and sixth decades of life. Fifteen to twenty percent of cases will present in prepubertal children. Approximately 10% will metastasize with metastasis more commonly seen in older patients. LCTs are immunoreactive with inhibin. Inhibin is a glycoprotein belonging to the TGF $\beta$  family. It is positive in a cytoplasmic distribution in LCTs as well as other sex cord-stromal tumors. LCTs will also express melan A, calretinin, and vimentin (Table 40.3). Calretinin, a calcium-binding protein, has a very similar staining profile as  $\alpha$ -inhibin for the sex cord-stromal tumors. However, while almost all LCTs are positive, only a minority of Sertoli cell tumors (SCTs) is immunoreactive. Cytokeratins and S-100 protein are either negative or only focally positive. CD30, CD117, OCT3/4, and PLAP are negative [30, 40, 48, 49]. Steroidogenic factor 1 (SF-1) is a nuclear transcription factor expressed in testicular Sertoli cells as well as other sex cord-stromal cells [50, 51]. While it appears to be expressed in a high percentage of sex cord-stromal tumors independent of type and eventually may prove to be a better marker than inhibin and calretinin, we need more studies to be performed before we advocate its routine use in lieu of the other markers.

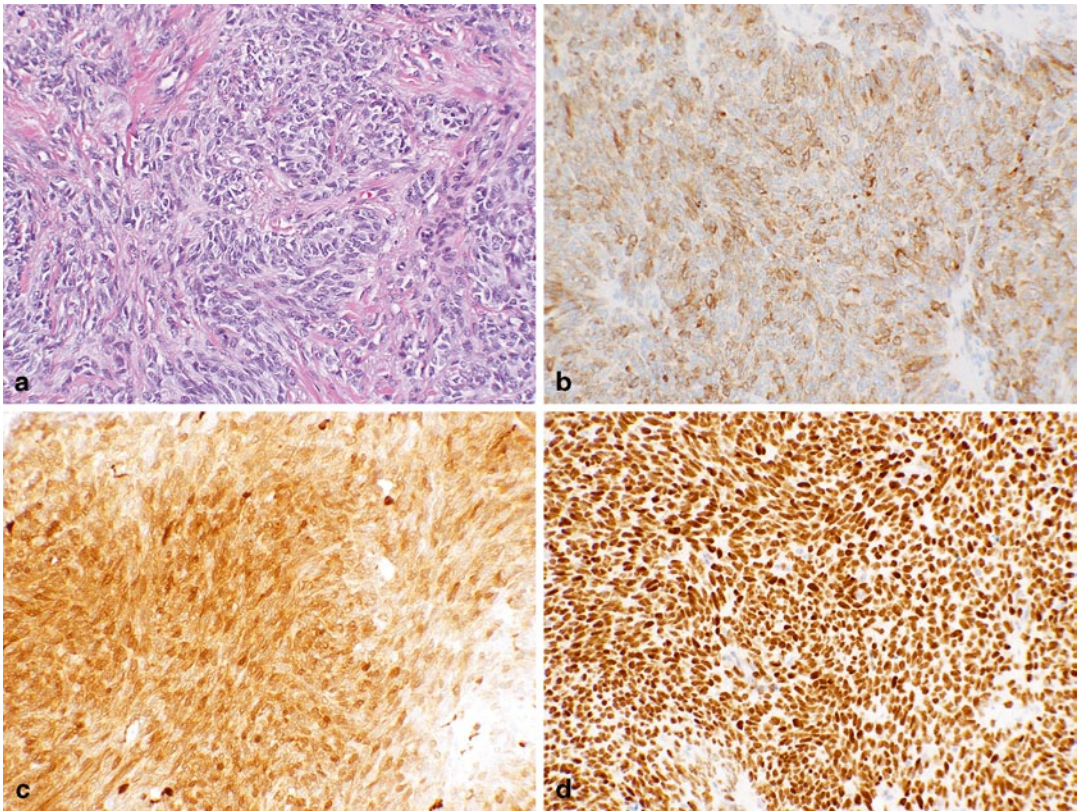
### Sertoli Cell Tumor: Usual Type and Its Variants

SCTs are rare, comprising less than 1% of testicular neoplasms [30, 52]. They may occur at any age and approximately 15% develop in children, but rarely before the age of 10 years [53]. SCTs are immunoreactive for SF-1 and vimentin, while staining for cytokeratin, inhibin, and calretinin is variable. Cytokeratins and EMA are more likely to be expressed in LCT than in other types of sex cord-stromal tumors. Calretinin cannot be used in isolation since it will also be expressed in adenomatoid tumor and mesothelioma. Markers typically seen in GCTs are negative [30]. The immunophenotype of the sclerosing variant of SCT as well as the large cell calcifying Sertoli cell tumor expresses the same immunophenotype as the usual type, even though the morphology of the latter is quite distinct (Table 40.3).

### Granulosa Cell Tumor

There are two subtypes of granulosa cell tumor, adult and juvenile, similar to what is seen in the ovary. The adult variant of granulosa cell tumor very rarely develops in the testis with less than 30 bonafide cases reported in the literature [47, 54, 55]. They have been described in males between the ages of 21 and 73 years and usually present as a testicular mass which may have been present for several years.

Juvenile granulosa cell tumors are the most common sex cord-stromal tumor of the infantile



**Fig. 40.9** Granulosa cell tumor. **a** H&E stain. **b** Inhibin. **c** Calretinin. **d** FoxL1 exhibits strong and diffuse nuclear immunoreactivity

testis [32, 56, 57]. They are usually encountered in the first 6 months of life, with one isolated case reported in a 21-month old and another in a 4-year old. Two cases have developed in undescended testes. Juvenile granulosa cell tumors may arise in patients with an abnormal karyotype and ambiguous genitalia.

The immunophenotype of granulosa cell tumors is similar to other gonadal stromal tumors. The cells of the adult type will express inhibin, WT-1, and SF-1. FOXL2 is a recently described marker which is expressed in most ovarian granulosa cell tumors. Although there is limited data in testicular primaries, it appears that up 40% will be positive while other types of gonadal stromal tumors are negative (Fig. 40.9a–d) [58, 59]. In the juvenile variant, the cell lining the microcysts may be cytokeratin positive while the surrounding stromal cells are positive for inhibin.

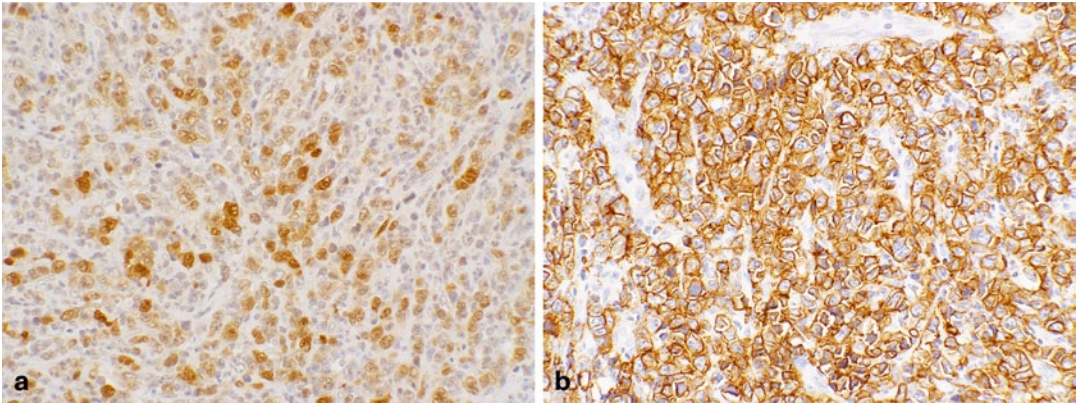
### A Rational Approach to the Workup of Testicular Tumors

In the preceding paragraphs, we have given a comprehensive list of all markers that have been used in the classification of testicular neoplasms. Clearly, it is impractical and borderline irresponsible to indiscriminately order these assays without taking into consideration the differential diagnosis suggested by the clinical scenario and the morphological features of the tumor. There are several scenarios in which a pathologist is likely to require ancillary studies.

### Is This Metastatic Tumor of Germ Cell Origin?

As always, a good clinical history and attention to the morphology of the tumor is paramount. If





**Fig. 40.10** Malignant lymphoma involving the testis. Notice nuclear expression of OCT3/4 (a) and CD20 (b)

fresh tumor is available, the most specific manner to establish the presence of *i(12p)* is by karyotype. If only FFPE tissue is available, one can submit the sample for FISH analysis, with the caveat that this assay is neither entirely sensitive nor specific. If immunohistochemical workup is required, a panel consisting of OCT4, D2-40, CD30, and Glypican 3 should suffice.

### Is This Testicular Tumor of Germ Cell Origin?

If testicular parenchyma is present, it is important to evaluate for the presence of IGCNU. If any doubt exists, OCT3/4, together with either D2-40 or CD117, will help to identify the neoplastic cells (Table 40.2). Within the invasive tumor, the stem cell marker OCT3/4 is positive in seminoma and EC, but may very rarely be expressed in other primitive tumors; we have seen nuclear immunoreactivity in a few carcinomas as well as in an anaplastic lymphoma (Fig. 40.10a,b). Besides OCT3/4, seminoma should be positive for CD117 and D2-40 and negative for CD30. EC is positive for OCT3/4 and CD30 but negative for CD117, D2-40, and Glypican 3. YST lacks immunoreactivity for OCT3/4, CD-30, CD117, and D2-40, but is positive for AFP and Glypican 3. We find SALL4 to be of limited utility in this differential diagnosis.

Interestingly, the morphology of YSTs can be even more varied in a setting of prior systemic

therapy or in late recurrences. In this situation, there is some evidence that Glypican 3 is the best way to identify YST lineage, and even to distinguish if one is dealing with a secondary somatic malignancy of germ cell origin or an unusual morphologic manifestation of YST. While this may be true, one must remember that the epithelial lining of some teratomas can express both AFP and Glypican 3.

### Is This Testicular Tumor of Sex Cord-Stromal Origin?

As strictly defined, intratubular germ cell neoplasia will be absent. All variants should be negative for the traditional germ cell markers (OCT3/4, SALL4, D2-40, CD117, CD30, Glypican 3); however, there is generally no need to perform all these assays (Table 40.3). Absence of SALL4 and Glypican 3 should suffice. In this setting, SALL4 may be better than OCT3/4 simply because its expression covers a wider distribution than OCT3/4 that would be expected to be negative in YSTs. Positive markers will vary somewhat between different types of sex cord-stromal tumor being considered, but all should express inhibin, WT-1, calretinin, and SF-1. Cytokeratin is of limited utility but is more likely to be expressed in SCTs. FOXL2 expression appears to be limited to granulosa cell tumors.

### Is This Tumor Arising in the Testicular Adnexa (Rete Testis, Epididymis) or the Mesothelial Lining?

Tumors that arise from the testicular adnexa are usually adenocarcinomas. Given their embryological origins, they are likely to express PAX8 as well as other markers typical for adenocarcinomas, such as CK7 and CEA. An unusual variant thought to arise from Müllerian rests will express PAX8 as well as estrogen receptor (ER). Mesotheliomas arising in the tunica vaginalis may involve the testicular parenchyma. These tumors express the same immunophenotype as mesotheliomas arising at any other site, including calretinin, and WT1, but not PAX8. None of these tumors should express markers associated with GCT lineage although the overlap with sex cord-stromal tumors, particularly calretinin in mesotheliomas, should be kept in mind.

### Is This Tumor a Lymphoma?

Malignant lymphoma represents up to 5% of testicular neoplasms. It is the most common bilateral tumor (either synchronous or metachronous) and the most common testicular tumor in men above the age of 60 [60]. The majority of patients have localized disease, but in a third of cases, testicular involvement is part of either regional or systemic disease. Lymphomas will express hematopoietic markers in accordance with their line of differentiation. They will not express cytokeratin or PLAP. We have seen a single large cell lymphoma with focal nuclear immunoreactivity for OCT3/4, but other GCT markers are negative while CD20 is positive (Fig. 40.10a, b). It is worth remembering that anaplastic large cell lymphoma is likely to express CD30, similar to EC and that we have encountered one case of anaplastic lymphoma with nuclear reactivity for the stem cell marker OCT3/4.

Primary leukemic tumors (granulocytic/myeloid sarcoma) of the testis are very rare [61]. Leukemic infiltration of the testis is most commonly seen on biopsy specimens in patients being

evaluated for relapse after systemic therapy [62, 63]. However, it may be seen at autopsy in up to 65–30% of patients with acute leukemia and chronic leukemia, respectively. Symptomatic enlargement of the gonad is encountered in 5% of cases. On microscopy, leukemic cells infiltrate between the seminiferous tubules and rarely extend into the seminiferous tubule itself. Marker expression will include MPO, lysozyme, CD68, and CD117, although the precise expression pattern will depend on the precise cell lineage. Common germ cell markers will be negative.

### Is This Tumor Metastatic Disease?

Metastasis to the testis from solid tumors is rare and usually presents in patients with known primary disease elsewhere and known metastatic disease. It is typically encountered in patients beyond the age of 50 years. The most common primary sites include prostate, colon, kidney, and melanoma [64]. In children, the most common tumors to metastasize to the testis include neuroblastoma and rhabdomyosarcoma [65–67]. In difficult cases, an immunohistochemical panel which includes a broad spectrum of entities may be required, but there is no substitute for a detailed clinical history.

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