

# Solid Tumour Section

## Review

### Testis: Germ cell tumors

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#### Identity

##### Alias

Testicular cancer

#### Classification

Germ cell tumours comprise a heterogeneous group of neoplasms, which can be found at different, although restricted anatomical locations. In the testis three groups of germ cell-derived tumours are distinguished:  
I- teratomas and yolk sac tumours of infants;  
II- seminomas and nonseminomas of adolescents and adults;  
III- spermatocytic seminomas of the elderly.

These groups are defined by epidemiological characteristics, histological composition and chromosomal constitution (Table 1). Designation of tumours to these groups is clinically relevant because they require different strategies for treatment.

#### Clinics and pathology

##### Disease

Testicular germ cell tumours, teratomas and yolk sac tumours, seminomas and nonseminomas, carcinoma in situ (CIS), intratubular germ cell neoplasia undifferentiated (IGCNU) , testicular intratubular neoplasia (TIN) , spermatocytic seminomas.

Table 1. Overview of the histology of testicular germ cell tumours related to mean age, precursor cell, and characteristic chromosomal anomalies.

Age (yrs)	Histology	Precursor	Chromosome anomalies
0-5	teratoma yolk sac tumour	embryonic germ cell "	not found aneuploidy, and loss: 6q gain: 1q,20q,22
15-45:			
15-30	nonseminoma	primordial germ cell	aneuploidy, and
30	combined tumour	"	loss: 4,5,11,13,18,Y
30-45	seminoma	"	gain: 7,8,12p,X
50 and older	spermatocytic seminoma	spermatogonia/ spermatocyte	diploidy/aneuploidy, gain: 9

### Embryonic origin

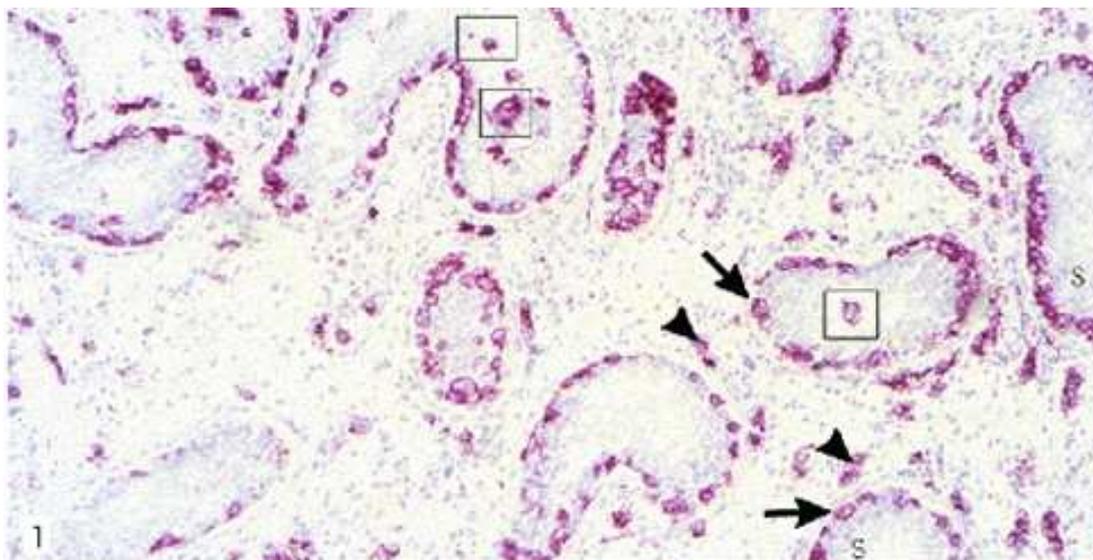
That the different types of germ cell tumours of the testis are derived from cells belonging to the germ cell lineage, is established, although the actual non-malignant counterparts are still a matter of debate. It is likely that the teratomas and yolk sac tumours of infants originate from an embryonic germ cell, while this is a spermatogonial/spermatocyte-type of cell for spermatocytic seminomas. In contrast, it is established that the precursor of seminomas and nonseminomas is carcinoma in situ (CIS), also referred to as intratubular germ cell neoplasia undifferentiated (IGCNU) or testicular intratubular neoplasia (TIN). CIS is composed of tumour cells located on the basal membrane at the inner side of the seminiferous tubules, under the tight junction, where normally the spermatogonia reside. It has been suggested that the normal counterpart of CIS, i.e., a primordial germ cell/gonocyte is present within the gonad around the 7th to 10th week gestational age. This is supported by the epidemiological finding that the incidence of seminomas and nonseminomas show a lower incidence in cohorts of men born during the period of the second world war in Denmark, Norway and Sweden. An alternative model has been proposed, in which the cell of origin is a pachytene spermatocyte.

### Epidemiology

During the first few years of life, the only types of germ cell tumours diagnosed in the testis are teratomas and yolk sac tumours. They are evidently unrelated to puberty. In contrast, the seminomas, nonseminomas,

and spermatocytic seminomas are clinically manifest during or after puberty, therefore likely related to sexual maturation. Spermatocytic seminomas are predominantly found in patients of 50 years and older. While most patients with a seminoma present in their 4th decade of life, this is in the 3rd decade for patients with a nonseminoma. An increasing incidence (in between 6-11/100.000) has been reported both for seminomas and nonseminomas during the last decades in white populations throughout the world, with an annual increase of 3-6%. Although in general rare, accounting for 1-2% of all malignancies in males, seminomas and nonseminomas are the most common cancer in young Caucasian males. In some European countries, i.e., Denmark and Switzerland, the life time risk for seminoma or nonseminoma is up to 1%. However, the increase seems to stabilise to date. In contrast to whites, blacks have a significantly lower, not increasing, incidence for seminomas/nonseminomas, although histology and age-distribution are the same. No significantly increasing incidence has been reported for teratomas and yolk sac tumours of infants and spermatocytic seminomas.

The incidence of CIS, the precursor of both seminomas and nonseminomas, in the general population is similar to the life time risk to develop a seminoma/nonseminoma. This indicates that CIS will always progress to invasiveness. About 5% of patients with a unilateral seminoma or nonseminoma have contralateral CIS.



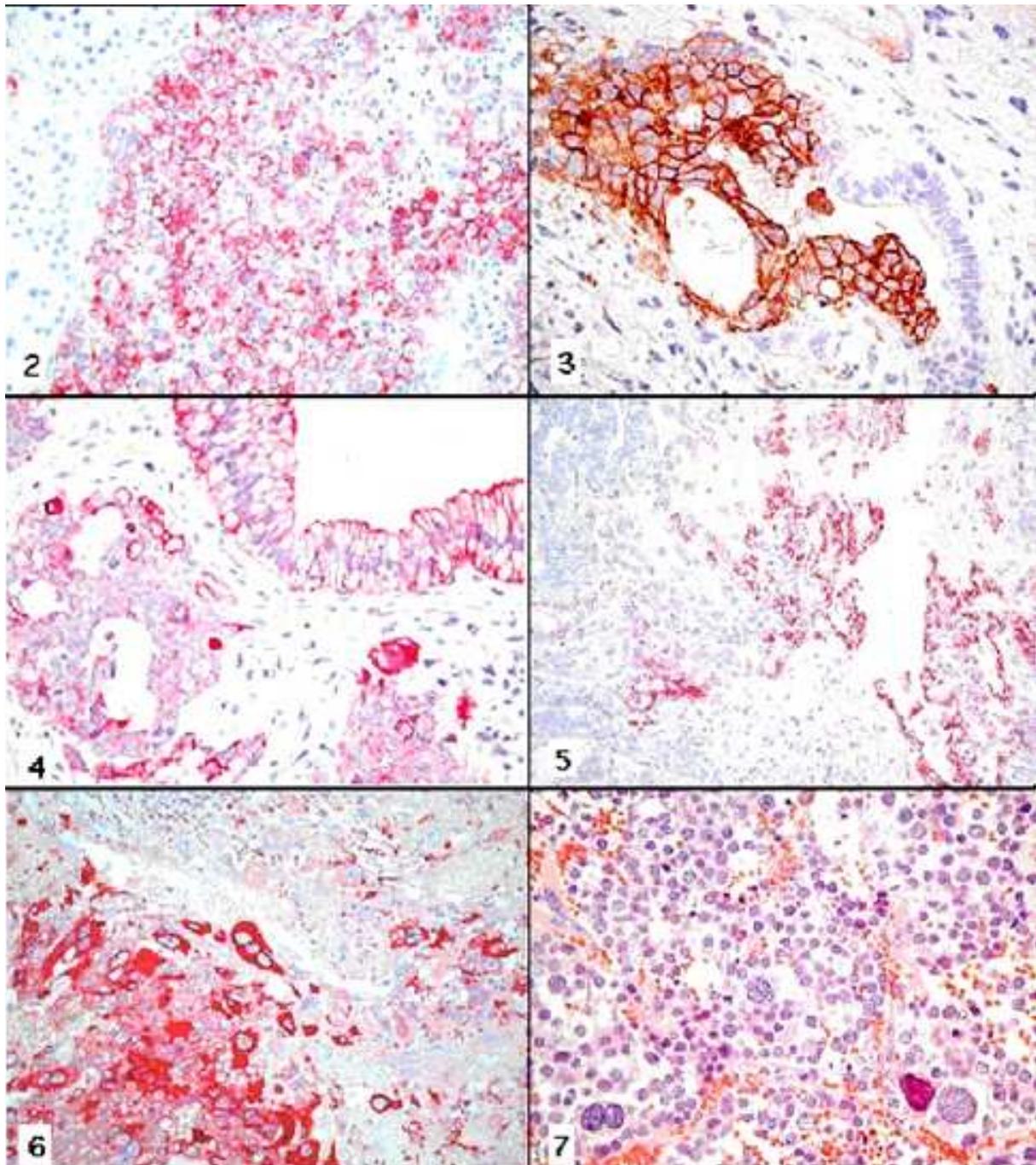


Figure 1. Representative example of the precursor cells of both seminoma and nonseminoma of the adult testis, known as carcinoma in situ (CIS), intratubular germ cell neoplasia undifferentiated (IGCNU), and testicular intratubular neoplasia (TIN). The cells are identified by detection of alkaline phosphatase reactivity on a frozen tissue section of testicular parenchyma adjacent to an invasive seminoma. Note the presence of the alkaline phosphatase positive IGCNU cells at the inner basal membrane of the seminiferous tubules (indicated by an arrow), under the tight junctions present between the Sertoli cells (indicated by 'S'). Micro-invasive seminoma cells (indicated by an arrow-head) are also detectable, as well as IGCNU cells in the lumen of the seminiferous tubules (within the squares).

Figure 2. Representative example of a seminoma, stained for placental/germ cell specific alkaline phosphatase. Note the presence of lymphocytic infiltrations.

Figure 3. Representative example of an embryonal carcinoma, stained for CD30.

Figure 4. Representative example of a teratoma, stained for cyokeratin.

Figure 5. Representative example of a yolk sac tumor, stained for AFP.

Figure 6. Representative example of a choriocarcinoma, stained for hCG.

Figure 7. Representative example of a spermatocytic seminoma, stained with hematoxylin and eosin.

## Pathology

CIS cells show similarities to embryonic germ cells, like their positivity for alkaline phosphatase, the stem cell factor receptor (c-KIT), and their glycogen content. These cells are frequently found in the adjacent parenchyma of an invasive seminoma and nonseminoma, of which a representative example is given in Figure 1. Histologically and immunohistochemically, seminoma cells mimic CIS. Lymphocytic infiltrations in the supportive stroma are a consistent feature of these tumors (Figure 2). So far, no differences have been found between CIS and seminoma cell, except the invasive growth of the latter. In contrast to the homogeneity of CIS and seminomas, nonseminomas can be composed of different elements, including embryonal carcinoma (the undifferentiated, stem cell, component), teratoma (the somatically differentiated component), yolk sac tumour and choriocarcinoma (the components of extra-embryonal differentiation) (see Figure 3-6). These different histological elements can be identified using immunohistochemistry for different markers, like CD30 for embryonal carcinoma, alpha fetoprotein (AFP) for yolk sac tumour, and human chorionic gonadotropin (hCG) for choriocarcinoma (see illustrations). Most nonseminomas are mixtures of these different elements. About 50% of germ cell tumors of adolescents and adults are pure seminomas, and 40% pure or mixed nonseminomas. Tumours containing both a seminoma and a nonseminoma component are classified as combined tumours according to the British Classification system, and as nonseminomas according to the World Health Organisation (WHO) classification. These tumours present at an age in between that of pure seminoma and nonseminoma.

The spermatocytic seminomas are histologically uniform and composed of three cell types, small, intermediate and large cells, that are evenly distributed (Figure 7). The immunohistochemical markers for CIS/seminoma are overall negative in spermatocytic seminomas. So far, no specific markers have been reported for spermatocytic seminomas. Histologically and immunohistochemically, the teratoma and yolk sac tumour components found in the infantile testis are indistinguishable from those elements found in nonseminomas of the adult testis. However, they differ in chromosomal constitution (see Table 1 and below), and the first lack CIS in the adjacent parenchyma.

## Evolution

In spite of the fact that it is generally accepted that CIS

is the precursor for seminoma and nonseminoma, the relationship(s) between these histological elements is still a matter of debate. It has been shown, especially using cell lines derived from nonseminomas, that embryonal carcinoma is the undifferentiated stem cell of all differentiated nonseminomatous components. So far, no cell lines for seminoma or CIS are available. Nonseminomas mimic embryonal development to a certain level. However, it is unproven so far whether seminomas may also progress into nonseminoma, although various observations, both biological and clinical, may support this model. It has been suggested that CIS present in the adjacent parenchyma of an invasive seminoma or nonseminoma is only one step behind in the progression of the cancer, which is supported by molecular findings.

## Prognosis

The teratomas of infants, and the spermatocytic seminomas are generally benign. Therefore, orchidectomy alone is mostly curative. However, spermatocytic seminomas may progress to sarcoma, a highly malignant tumour. When the yolk sac tumour component of infants is metastatic, it can be cured in the majority of patients using chemotherapy. Seminomas are highly sensitive to irradiation, while nonseminomas are overall highly sensitive to cisplatin-based chemotherapy, with cure rates of up to 90%. Criteria have been developed to distinguish nonseminoma patients with a good, intermediate and poor response (Table 2). Although these parameters are not informative on an individual basis, they separate the three groups as a whole. Seminoma patients always fall in the good and intermediate prognostic group. Stage I disease might be treated by orchidectomy followed by a "wait and see" strategy. Alternatively, retroperitoneal lymph node dissection (nerve sparing) and/or irradiation (in case of pure seminoma) can be performed. Moreover, a single dose cisplatin-based chemotherapy is tested in an experimental set up. These issues are of interest, because the risk of occult metastases in clinically stage I nonseminomas is about 30%. Established factors predicting metastatic disease are lymphovascular space invasion and percentage of embryonal carcinoma. For nonseminomas there is no consensus on the best method to define the risk of occult metastases and on how the information can be used for the clinical management of patients. In clinically stage I seminoma patients occult metastases are predicted by vascular invasion and tumor size. More recently, the mean nuclear volume has been reported to be an informative parameter.

Table 2 Risk stratification from the international germ cell cancer collaborative group

	Serum marker			Extent of disease
	LDH (times normal)	hCG (mIU/mL)	AFP (ng/mL)	
<b>Nonseminomas:</b>				
Good risk:	1.5	< 5000	<1000	no nonpulmonary metastases
Intermediate risk:	1.5-10	5000-50,000 or 1000-10,000		no nonpulmonary metastases
Poor risk:	>10	>50,000	>10,000	nonpulmonary metastases
<b>Seminomas:</b>				
Good risk:				no nonpulmonary metastases
Intermediate risk:				nonpulmonary metastases

Obviously these parameters could serve to define a group of patients that could benefit from surveillance. Patients with refractory disease might benefit from high-dose chemotherapy.

Because CIS is formed during intra-uterine growth, and the treatable cancer in most cases becomes clinically manifest after puberty, methods for early diagnosis and treatment might prevent progression of CIS to an invasive seminoma or nonseminoma, thereby preventing possible progression to refractory disease. A number of putative parameters have been reported, although none of them have been tested in a clinical setting thus far. Moreover, it has been shown that CIS can be effectively eradicated using local irradiation, with limited side effects.

The presence of CIS in the contralateral testis in 5% of patients with a seminoma or nonseminomas has led to the routine of a contralateral biopsy in some countries. However, in most countries the clinicians prefer a closely "wait and see" strategy. Patients with cryptorchidism, atrophic testis, or prior infertility have a higher risk of CIS in the contralateral testis. The exact numbers are unknown, but it is estimated that high-risk patients comprise 40-50% of the population with CIS. Altogether about 50-60% of patients with a unilateral testis tumor will have no other risk factors for CIS.

## Genetics

### Note

Familial predisposition About 2% of the patients with a seminoma or nonseminoma have an affected family member, indicating a genetic component in the development of this cancer. So far, two genome-wide linkage analyses have been performed. The first showed linkage to regions of chromosome 1, 4, 5, 14 and 18, while the second found no evidence for linkage to chromosome 1, and a weaker indication for involvement of region 2 of chromosome 4 (4cen-q13). For the other region on chromosome 4 (p14-p13), and for chromosome 5, similar results were obtained. Both

studies indicated linkage to chromosome 18. The latter study found linkage to the short arm of chromosome 2, and the telomeric region of 3q. A telomeric region of the long arm of chromosome 12 showed linkage when the results of both studies were combined, while no linkage was found in the separate studies. An other finding of interest is the fact that bilateral occurrence of the tumor is more frequent in familial than in sporadic cases (15 versus 5%). Indeed, most recently linkage to Xq27 has been found for cryptorchidism and bilateral germ cell tumors, although the gene involved is still unknown.

## Cytogenetics

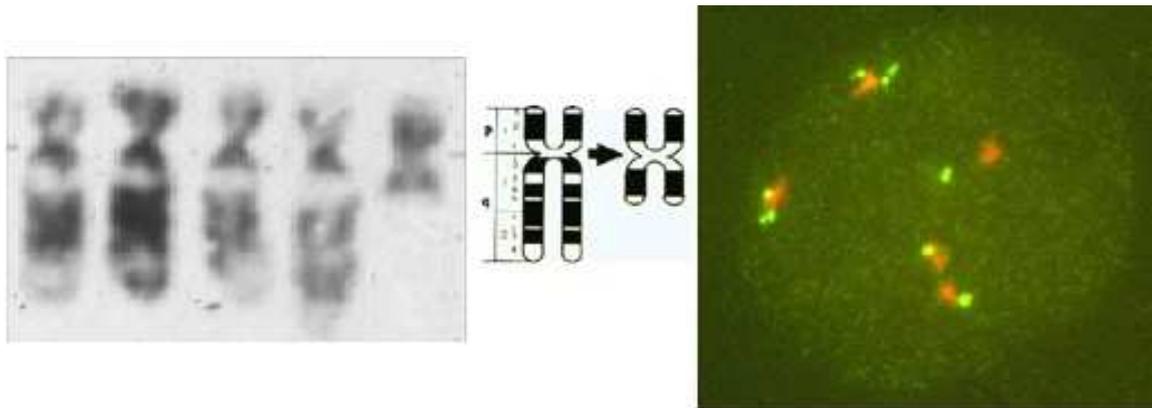
### Cytogenetics Morphological

The three groups of germ cell tumours of the testis show characteristic chromosomal anomalies, which favor the model of separate pathogeneses. The chromosomal data on germ cell tumors of the infantile testis and spermatocytic seminomas are scarce.

While no aberrations are found so far in teratomas of the infantile testis, the yolk sac tumours show recurrent loss of part of 6q, and gain of parts of 1q, 20q, and 22. In addition, these yolk sac tumours are all found to be aneuploid.

One study reports the analysis of spermatocytic seminomas by karyotyping and comparative genomic hybridization, showing gain of chromosome 9 as the only recurrent and characteristic chromosomal abnormality.

Seminomas, nonseminomas as well as CIS are consistently aneuploid with a characteristic pattern of chromosomal gains and losses. The cells of seminoma and CIS are hypertriploid, while those of nonseminoma, irrespective of histological composition, are hypotriploid. Using karyotyping, more recently supported by in situ and comparative genomic hybridization, a complex, but similar pattern of over- and underrepresentation of (parts of) chromosomes has been identified in seminomas and nonseminomas.



Representative example of: actual G-banding and schematic of a normal chromosome 12 (left within panel) and an isochromosome 12p (i(12p)) (right within panel); the fluorescent in situ hybridization pattern with a probe specific for the centromeric region of chromosome 12 (red) and the p-arm (green). Note the presence of three normal chromosomes 12 (paired green and red signal), and two isochromosomes (one red and two green signals).

Overall, the chromosomes 4, 5, 11, 13, 18 and Y are underrepresented, while the chromosomes 7, 8, 12 and X are overrepresented. In spite of the highly similar pattern of gains and losses in seminomas and nonseminomas, some differences were observed, like overrepresentation of chromosome 15 in seminomas compared to nonseminomas, which might explain the ploidy difference between these two histological groups.

The recurrent pattern of chromosomal gains and losses suggests that both activation of proto-oncogenes, and inactivation of tumor suppressor genes is involved in the development of this cancer.

**Gain of 12p** The isochromosome 12p can be used as a diagnostic molecular marker for seminomas and nonseminomas: the most consistent chromosomal anomaly in seminomas and nonseminomas, besides their aneuploidy, is gain of the short arm of chromosome 12. In fact, about 80% of the invasive tumors have extra copies of 12p due to the formation of an isochromosome (i(12p)) (Figure). The 20% i(12p) negative tumors also show gain of 12p, due to other chromosomal changes. These data strongly indicate that the short arm of chromosome 12 contains a gene or genes of which extra copies are required for the development of the invasive tumor. Analysis of LOH on the long arm of chromosome 12 showed that polyploidisation occurs prior to i(12p) formation. In addition, it was demonstrated that i(12p) results from sister chromatid exchange. In contrast, non-sister chromatid exchange has also been suggested. Most recently, it was shown that the presence of extra copies of the short arm of chromosome 12 is related to invasive growth of the tumor, i.e., no gain of 12p is observed in CIS. This suggests that addition copies of one or more genes on 12p is relevant for the progression of CIS to an invasive tumor. Analysis of seminomas and nonseminomas containing a high level amplification of a restricted region of 12p, i.e., band p11.2-12.1, cyclin D2 being outside this region, might

be a tool to identify the gene(s) on 12p. So far, these data suggest that it relates to a gene that suppresses induction of apoptosis upon invasive growth of the tumour cells.

**Proto-oncogenes** Several studies deal with the possible role of activation of proto-oncogenes in the development of seminomas and nonseminomas. RAS genes are rarely found to be mutated. One study reported the presence of mutations in c-KIT in some cases. Overexpression of c-MYC has been found in less than 10% of nonseminomas, and amplification of MDM2 in also less than 10% of the tumors. Cyclin D2 has been suggested as the candidate gene on 12p. However, this gene maps outside the amplified region found in some seminomas and nonseminomas. In conclusion, the role of activation of proto-oncogenes in the genesis of seminomas and nonseminomas is not elucidated so far.

**Tumor suppressor genes** Studies of loss of heterozygosity (LOH), a hallmark of the involvement of tumor suppressor genes, have given rather inconsistent results in seminomas and nonseminomas, which might be related to their aneuploidy. Several studies have been performed on chromosomes 1, 5, 11, 12 and 18. Recurrent loss has been observed on 1p, in particular bands p13, p22, p31.3-p32, and 1q; in particular bands q32. Several regions on chromosome 5 show LOH, including p15.1-p15.2, q11, q14, q21, and q34-qter. Chromosome 12 contains two regions of interest, i.e., q13 and q22. In spite of the identification of homozygous deletions at 12q22, no candidate genes have been identified so far. Homozygous deletions have also been identified on the long arm of chromosome 18. Although DCC (deleted in colorectal cancer) might be a candidate, it has been indicated that loss of this gene is likely progression-related. More recently, inactivating mutations of SMAD4, also mapped to 18q, have been reported in a limited number of seminomas. LOH analysis on microdissected tumor cells of different histologies, including CIS, revealed

recurrent LOH at 3q27-q28, 5q31, 5q34-q35, 9p21-p22 and 12q22. These anomalies were also found in the adjacent CIS cells. Interestingly, loss of 3q27-q28 was only but consistently detected in the embryonal carcinoma components. The other targets investigated with overall negative findings are: NME1 and 2, APC, MCC, RB, WT1, and P53. However, hypermethylation of exon 1 of p16 was found in about 50% of the tumors, which was related to no, or a low level of expression.

In summary, although interesting observations have been made, no convincing data based on studies on LOH, mutations and expression so far, indicate a significant involvement of one of the studied tumor suppressor genes in the development of testicular seminomas and nonseminomas. Moreover, no candidate genes have been identified for the teratomas and yolk sac tumors of the infantile testis, as well as for spermatocytic seminomas.

### Cytogenetics Molecular

The isochromosome 12p can be identified on interphase nuclei by fluorescent in situ hybridization, using simultaneously a probe specific for the centromeric region and the short arm of chromosome 12. The use of the centromeric probe only was not found to be informative.

## Genes involved and proteins

### Note

In spite of several suggestions about a possible role of a number of genes in the development of teratomas and yolk sac tumours of the infantile testis, the seminomas and nonseminomas and the spermatocytic seminomas, actual prove for their involvement is missing so far.

## To be noted

### Note

Gain of 12p is restricted to invasive seminomas and nonseminomas, and is not found in CIS. Therefore additional copies of the gene(s) on 12p is not involved in the early development of this cancer.

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