The term ‘germ cell tumours’ refers to a heterogeneous group of neoplasms that originate from cells belonging to the germ cell lineage. They occur mainly in the gonad, but also in some specific extragonadal sites. This distribution has been explained by the migration route of primordial germ cells from the yolk sac to the genital ridge. Although the gonad, the final destination of germ cells, is the most frequent anatomical localization of germ cell tumours, germ cell tumours can also be found in the sacral area, mediastinum, head and neck and hypothalamus–pineal gland region, and very rarely in various organs not in the midline of the body.

The clinical behaviour of germ cell tumours depends on the sex and age of the patient, and the anatomical localization and histological composition of the tumour. The more benign variants, showing only somatic differentiation, known as mature teratoma, are found mainly in the ovary (dermoid cyst) and in extragonadal sites, the infantile testis and in the head and neck and sacrococcygeal region. These tumours may give rise to secondary malignancies. In contrast, the malignant germ cell tumours, predominantly found in males, are mainly identified in the adult testis, and sometimes in the anterior mediastinum and hypothalamus–pineal gland region. These tumours are characterized by the fact that they may contain the neoplastic counterparts of early germ cells, known as seminomas in the testis and the anterior mediastinum, dysgerminoma in the ovary, and germinoma in the midline of the brain. In addition, a histologically separate group of germ cell tumours, known as spermatocytic seminomas, can be found in the adult testis. Testicular germ cell tumours of adolescents and adults (TGCTs) have drawn most attention because they are relatively frequent. Moreover, TGCT is the first and only solid tumour of adults that responds well to chemotherapy. The germ cell tumours of the infantile testis and spermatocytic seminomas are less well studied because of their rarity; however, significant progress has been made in the understanding of their pathogenesis. In this review, the pathogenesis of these three different entities of germ cell tumours of the testis will be discussed.

Testicular germ cell tumours

Three epidemiologically, clinically and histologically defined entities of germ cell tumours can be distinguished in the human testis. The first group includes the teratomas–yolk sac tumours, which become manifest usually in the first 4 years of life and always before puberty. The second group, referred to as TGCTs, comprises the seminomas and nonseminomas,
which become manifest after puberty. Tumours of this entity presenting in boys with an early puberty are sometimes erroneously included in the infantile germ cell tumours. The third group comprises the spermatocytic seminomas, which usually appear in elderly men. Data will be presented indicating that these three entities have a different pathogenesis.

Epidemiology, histology and clinical behaviour

The testicular teratomas–yolk sac tumours (with an incidence of about 0.12 per 100 000, with a 1:4 ratio) are found in neonates and infants, well before puberty. Therefore, their development is not influenced by factors involved in the initiation and maintenance of spermatogenesis. In contrast, the development of TGCTs and spermatocytic seminomas is associated with spermatogenesis. Although germ cell tumours are rare in the general male population, accounting for < 1% of all cancers, the TGCTs are the most common malignancy in young adult Caucasian males. They are found mainly in the third and fourth decade of life. To date, the incidence of TGCTs is between 6 and 11 per 100 000, and it is increasing (Bergström et al., 1996). A similar pattern has not been reported for the infantile testis tumours and spermatocytic seminomas. Spermatocytic seminomas have an incidence of about 0.2 per 100 000, and occur mainly in elderly men (Burke and Mostofi, 1993).

Germ cell tumours of the infantile testis are composed of teratoma or yolk sac tumours. The teratomas are benign and can be cured by orchidectomy. The yolk sac tumours are malignant and may require additional chemotherapy, in particular in infants > 1 year of age. Spermatocytic seminomas have a striking morphology, showing characteristics of spermatogonia stage B, without expression of alkaline phosphatase and c-KIT (Rosenberg et al., 1997, and references cited therein). Although they are benign and can be cured using orchidectomy, they can very rarely give rise to sarcomatous elements (Burke and Mostofi, 1993).

It is now generally accepted that TGCTs originate from carcinoma in situ (CIS), which is also known as intratubular germ cell neoplasia, first described by Skakkebæk (1972). It has been suggested that CIS cells occur in the infantile testis adjacent to teratomas–yolk sac tumours. However, this is still a matter of debate. In contrast, it is generally agreed that spermatocytic seminomas originate from a later stage of germ cell development, and that they are not accompanied by CIS. A spermatocytic seminoma is usually accompanied by an intratubular spermatocytic seminoma, probably the preinvasive precursor of this neoplasm. CIS cells can be found in the adjacent parenchyma of most TGCTs, and also before the development of an invasive TGCT (for review, see Ulbright, 1993). Eventually, CIS will progress to invasiveness. Spontaneous regression does not seem to occur (Giwercman et al., 1991a). CIS cells are localized in the seminiferous tubules between the basal membrane and the Sertoli cell layer. They resemble primordial germ cells phenotypically (Jorgensen et al., 1995), for example, sharing expression of alkaline phosphatase (Box 1) and the stem cell factor receptor, c-KIT. The enzymatic activity of alkaline phosphatase isozymes can be used as a marker to identify CIS cells, using a relatively simple approach on frozen tissue sections (Fig. 1; Box 1). Epidemiological data support the hypothesis that the initiating event in the development of TGCTs occurs during intrauterine development (Møller, 1989). The finding that neonatal jaundice and low and high birth weights are associated with an increased risk for the development of this cancer (for review, see Swerdlow, 1997) indicates that tumour development starts very early, most likely in prenatal life. These observations may be related to oestrogen exposure during pregnancy. Other risk factors are gonadal dysgenesis, cryptorchidism, familial predisposition, childhood hernia and testicular atrophy. Less important factors are age at puberty, exercise, social class and sedentary life. These factors do not seem to influence the development of the germ cell tumours of the infantile testis and spermatocytic seminomas. However, the epidemiological data on these rare tumours are too limited to be certain.

Histologically and clinically, TGCTs are divided into seminomas and nonseminomas (for review, see Ulbright, 1993). The seminomas are composed of cells similar to CIS cells. Lymphocytic infiltrations in the supportive stroma are a consistent feature of these tumours. The undifferentiated stem cells of nonseminomas are termed embryonal carcinoma cells, and can differentiate into a broad spectrum of somatic tissues (teratomas) and the extra-embryonal derivatives: yolk sac tumours and choriocarcinomas. Most nonseminomas are mixtures of these different elements. In summary, CIS and seminoma cells show the characteristics of primordial germ cells. The development of nonseminomas resembles embryonal development to a certain extent, and this is illustrated by the presence of embryoid
bodies in nonseminomas, structures that mimic those of day 10 of embryonal development. In fact, nonseminomas and derived cell lines are used to study mechanisms involved in early development.

Histologically, TGCTs can be divided into the pure seminomas (about 50%) and nonseminomas (40%), which can be either pure or mixtures of the different nonseminomatous elements. The remaining 10%, containing both a seminoma and a nonseminoma component, are classified as combined tumours according to the British system (Pugh, 1976). The mean age of patients presenting with a seminoma is around 35 years. Patients with a nonseminoma present at a mean age of 25. The mean age of patients with a combined tumour lies in between.

About 80% of the patients with a seminoma have localized disease, which can be treated successfully using orchidectomy and surveillance, possibly combined with radiation therapy. Cisplatinum-based chemotherapy of patients with higher stage disease is usually curative. In spite of the success of cisplatinum-based therapy also for nonseminomas, about 20% of the patients with metastases die as a result of their tumour (Toner and Motzer, 1998). The response to therapy cannot be predicted with certainty in the group of poor risk patients. CIS cells can be effectively eradicated using a low dose of irradiation, resulting in prevention of the development of an invasive tumour with minor side effects (Giwercman et al., 1991b). It has been suggested that the treatment sensitivity of TGCTs, including CIS, is due to the presence of wild type P53 rendering the cells sensitive to treatment-induced apoptosis (Lutzker and Levine, 1996).

Genetic predisposition

A number of observations indicate that genetic factor(s) are involved in the development of TGCTs, for example, the large differences in the incidence of this tumour between Black and Caucasian men (Moul et al., 1994). The presence of higher testosterone concentrations during pregnancy in Black women compared with Caucasian women may be related to this phenomenon. It has been proposed that 25–33% of all TGCT patients have a genetic predisposition (Nicholson and Harland, 1995). About 2% of patients have an affected family member. These TGCT-prone families most often have only two affected members. No association with one of the known hereditary cancer syndromes has been demonstrated so far. Patients in TGCT-prone families are younger at clinical diagnosis than patients with a sporadic TGCT, and the tumour more frequently affects both testes (15% versus 5%). Patients with a bilateral TGCT have the same age at clinical presentation as familial cases.

The first genome-wide linkage analysis by sib-pair analysis in 35 TGCT-prone families (carried out by the Imperial Cancer Research Fund (ICRF)) showed linkage to chromosome 1, 4 (two regions), 5, 14 and 18 (Leahy et al., 1995). A second genome-wide study by the International Testis Cancer Linkage Consortium (ITCLC) in 54 new families found no evidence for linkage to chromosome 1, and a weaker indication for involvement of 4cen–q13 (International Testicular Cancer Linkage Consortium, 1998). For the other region on chromosome 4 (p14–p13), and for chromosome 5, similar results were obtained. Although analysis of additional ICRF families reduced the original LOD score for chromosome 4 in the ICRF study, the ITCLC study confirmed linkage to this chromosome (LOD score is a measure of the probability that the genetic linkage, defined as the log10 ratio of the probability that the data would have arisen if the loci are linked to the probability that the data could have arisen from unlinked loci). Both studies indicated linkage to chromosome 18, although the combined results weakened this association. The ITCLC study found linkage to the short arm of chromosome 2, and to the telomeric region of 3q. When the results of both studies were combined, linkage was found in the telomeric region of the long arm of chromosome 12, while no linkage was found in the separate studies.

In conclusion, linkage analysis indicates the involvement of several chromosomal regions in the development of TGCTs. However, more families need to be included before firm conclusions can be drawn. So far, no familial clustering has been reported for infantile germ cell tumours and spermatocytic seminomas.

Chromosomal constitution

Several techniques for investigating genomic aberrations (Boxes 2 and 3) have been applied to the different entities of testicular germ cell tumours. The most important findings will be discussed.

Teratoma–yolk sac tumours

Several studies have addressed the flow cytometry and karyotyping of teratomas–yolk sac tumours of the infantile testis. In general, teratomas are diploid (Hoffner et al., 1994; Silver et al., 1994; Stock et al., 1995). The yolk sac tumours may be aneuploid (mainly near-tetraploid) (Oosterhuis et al., 1989; Komnoss et al., 1990; Hu et al., 1992; Perlman et al., 1994; Silver et al., 1994; Jenderny et al., 1995). The structural abnormalities are: deletions of 1p, in particular region p36;
Pathogenesis of testicular germ cell tumours

Loss of 6q; structural anomalies of chromosome 2 and of the short arm of chromosome 3 (Perlman et al., 1994; Jenderny et al., 1995; Stock et al., 1995). One study reported the absence of aberrations affecting chromosome 12 (Perlman et al., 1994) and another report showed that gain of this chromosome occurs (Jenderny et al., 1995). A series of four teratomas of the infantile testis have been studied using comparative genomic hybridization (CGH) (M. Mostert, unpublished). Two of these teratomas showed no aberrations in microdissected samples with minimal contamination with normal cells and the other two showed gain of (parts of) 3p, 12p (12.1–13.1), 17, 19q, 20q, 22, and Xq, and loss of (parts of) 4, 6q, and 8q. These data support the published heterogeneity in the chromosomal constitution of teratomas of the infantile testis. Two pure yolk sac tumours were also studied, showing gain of (parts of) 9q, 14, 19q, 20q, and 22, and loss of (parts of) 4q, 5, and 6q. A recurrent yolk sac tumour of a sacral teratoma showed a number of similar imbalances, such as gain of (parts of) 3p, 17q, 19q, 20q, and 22, and loss of (parts of) 4, 6q, and 8q. Gain of (parts of) 19q, 20q, and 22, and loss of (parts of) 4q and 6q were present in all cases, strongly indicating a pathogenetic relationship. The malignant potential of yolk sac tumours is borne out by their aneuploidy.

Seminomas and nonseminomas

Flow cytometry and image analysis demonstrated that both seminomas and nonseminomas are consistently aneuploid.

**Box 2. Methods for detecting genomic aberrations**

Besides the detection of the loss of heterozygosity by Southern blot analysis, which will not be discussed here, several other approaches are available to obtain information about the genomic constitution of cells of interest. Flow cytometry and image analysis are suitable for identifying populations of cells with an aneuploid DNA content. These methods can be performed both on fresh and archival material (frozen and formalin-fixed, paraffin wax-embedded). Because total DNA content is measured, no information about the nature of the chromosomal aberrations will be obtained. In contrast, detailed information can be gathered using karyotyping. Because of the need for metaphase spreads, which can be obtained either by direct harvesting or by culturing the tumour cells in vitro, this approach can only be performed on fresh tissue. This may result in the selection of subclones. An additional limitation of this approach is the restricted amount of information that can be obtained from the banding patterns of the chromosomes (resolution of about 2–10 megabases), especially regarding those that are structurally aberrant (Fig. 2b,d). Molecular cytogenetic methods have been developed, including (fluorescent and non-fluorescent) in situ and comparative genomic hybridization (ISH and CGH). Fluorescent ISH can be performed in combination with karyotyping, allowing a more detailed analysis of the chromosomal aberrations present in a metaphase spread (Fig. 2e). In addition, this technique can be applied to interphase nuclei, to visualize numerical and structural anomalies. This particular application allows investigation of archival tissue. The most important limitation of ISH is the restricted amount of information that can be obtained within a single experiment, owing to the necessity to select targets for investigation. This problem has been solved by the development of the multi-colour fluorescent ISH, or SKY, method. Although of importance, this technique will not be discussed here, because, so far, it has not been applied to germ cell tumours. Fluorescent ISH can also be complemented by CGH, a method allowing the identification of relative gains and losses throughout the genome in a single experiment (see Box 3).
Box 3. Comparative genomic hybridization

Comparative genomic hybridization (CGH) is a modified fluorochrome in situ hybridization approach, in which DNA isolated from cells of interest is hybridized simultaneously with control DNA (either 46XY, or 46XX) on a normal metaphase spread (46XY or 46XX). Before hybridization, the DNA samples are differentially labelled so that they can be distinguished using fluorescent dyes, either directly or indirectly (see Fig. 3). Ratios beyond the thresholds between the fluorescent dyes (mostly 0.85 and 1.15, determined on the basis of normal/normal controls) are indicative for the chromosomal regions showing gains and losses. Because total DNA is used for this method, fresh, frozen or fixed material can be used, although the quality of fixed material can be a limiting factor. A recent modification is CGH on amplified DNA isolated from microdissected cells. This allows analysis of possible heterogeneity within a tumour, and the chromosomal constitution of different pathogenetic stages. The resolution of CGH is in principle similar to that of banding techniques. The major advantage of CGH is the fact that no bias due to in vitro or in vivo selection of subpopulations of cells is introduced, and an overview of the imbalances throughout the genome is obtained in a single experiment. However, aberrations that do not result in chromosomal gains and losses (like reciprocal translocations) go undetected. Because relative differences in copy numbers are identified compared with the total DNA content, CGH cannot determine DNA ploidy.

(Oosterhuis et al., 1989). The median DNA content of seminomas is hypertriploid, and that of nonseminomas is hypotriploid. This difference is also found in the seminoma and nonseminoma components of combined tumours. CIS in the adjacent parenchyma of both seminomas and nonseminomas has the same ploidy as seminomas (De Graaff et al., 1992).

In agreement with the data on DNA flow cytometry, seminomas have chromosome numbers in the hypertriploid and nonseminomas in the hypotriploid range. Karyotyping also showed a strikingly similar pattern of over- and under-representation of (parts of) chromosomes (for review, see Van Echten-Arends et al., 1995); chromosomes 7, 8, 12, 21, and X are over-represented, while chromosomes 11, 13, 18 and Y are under-represented. Several investigators have shown, using CGH, that these numerical changes are not due to in vitro or in vivo selection of particular subclones (Korn et al., 1996; Mostert et al., 1996; Ottesen et al., 1997; Summersgill et al., 1998). This finding indicates that nonrandom numerical aberrations of these chromosomes, most likely related to the presence of (proto-)oncogenes and tumour suppressor genes, do play a role in the development of this cancer. It remains to be shown whether these numerical changes are crucial for early development of this cancer, or whether they are related to progression. Karyotyping has also demonstrated consistent differences in the chromosomal constitution of seminomas and nonseminomas. In seminomas, (parts of) chromosomes 7, 15, 19 and X are over-represented compared with the equivalent chromosomes in nonseminomas (Van Echten-Arends et al., 1995). Use of in situ hybridization on frozen tissue sections confirmed this difference for chromosome 15 (Loojenga et al., 1993). In addition, it has been demonstrated that this difference in copy number of chromosome 15 was also present in the adjacent CIS cells. CIS adjacent to nonseminomas, in terms of copy numbers of chromosome 15, resemble nonseminomas, and yet are phenotypically similar to seminomas.

Short arm of chromosome 12. The demonstration of consistent structural chromosomal abnormalities is often the basis for the identification of (proto-)oncogenes and tumour suppressor genes involved in the development of a particular tumour type. The most consistent structural chromosomal aberration in TGCTs is an isochromosome of the short arm of chromosome 12 ([i(12p)] (Fig. 2). This anomaly was first described by Atkin and Baker (1982). About 50% of the seminomas and 80% of the nonseminomas show at least one i(12p) (Van Echten-Arends et al., 1995). It has been demonstrated that polyploidization precedes i(12p) formation (Geurts van Kessel et al., 1989). TGCTs without an i(12p), known as i(12p)-negative TGCTs, also show gain of 12p-sequences, demonstrated by fluorescent in situ hybridization (ISH) (Rodriguez et al., 1993). This consistent gain of 12p, supported by CGH (Korn et al., 1996; Mostert et al., 1996; Ottesen et al., 1997; Summersgill et al., 1998), indicates that it is crucial for the development of TGCTs. Isochromosome 12p has also been found in a small number of cases of CIS (Vos et al., 1990).

The short arm of chromosome 12 contains about 40 megabases, and may harbour 120 genes. Looking for candidate genes at the entire 12p region is not feasible. Therefore, the demonstration of amplification of a more restricted region (12p11.2–p12.1) of the short arm of chromosome 12 in a metastatic seminoma is of great interest (Suijkerbuijk et al., 1994), particularly since this region is more centromeric than the area over-represented in infantile testicular tumours. This specific aberration is now described in a small series of primary TGCTs, preferentially seminomas (Korn et al., 1996; Mostert et al., 1996). A molecular approach has been used to define the amplified region more precisely. After construction of a contig of the region of interest, double fluorescent ISH was used to show that the shortest region of overlap of amplification (SROA) is about 1.7 megabases (Mostert et al., 1998). Three known genes are located in the SROA: SOX5, JAW1 and K-RAS (Fig. 4). In view of the function of these genes, and their expression in TGCTs, K-RAS is a possible candidate. So far, 14 TGCTs with a 12p-amplification have been identified: eight seminomas, two combined tumours, and four nonseminomas (H. Roelofs, unpublished), accounting for about 5% of TGCTs. The majority of the tumour cells in seminomas contained the amplification. In nonseminomas, the pattern was more heterogeneous. Although the amplification has been demonstrated in microinvasive seminoma cells, it has not yet be identified in CIS cells. This finding indicates that amplification may be involved, as cause or effect, in invasive growth. The seminomas with amplification were clinically manifest at a significantly younger age (P < 0.05) than seminomas without amplification (H. Roelofs, unpublished). In fact, the seminoma patients with an amplification had the same mean age as patients with nonseminomas. Whether the presence of the amplification has prognostic or predictive significance is not known.

Chaganti and coworkers suggested that cyclin D2 (mapped to 12p13) is the most likely gene to explain gain of 12p-sequences in TGCTs (Houldsworth et al., 1997) and propose that TGCTs originate from a precursor cell at the pachytene
stage of meiosis (Chaganti and Houldsworth, 1998). So far, assays are lacking to prove that a particular candidate gene is of pathogenetic relevance.

Survival of seminoma cells in vitro. No cell lines of seminomas or CIS have been established. In our first attempts to grow seminoma cells in vitro, survival has been improved significantly by using rat Sertoli cells as a feeder layer (Berends et al., 1991). However, there was no cell proliferation, and the cells were not detected by day 10. Because of the phenotypic similarities between seminomas and primordial germ cells (see above), we have cultured a series of seminomas in vitro using conditions successfully used to culture mouse primordial germ cells (Matsui et al., 1992). In the majority of cases, there was again no proliferation and a rapid disappearance of cells owing to the induction of apoptosis upon disruption of the tumour microenvironment (Olie et al., 1996), a process known as anoikis (Frisch and Francis, 1994). However, a few seminomas could be cultured for a limited period of time (Olie et al., 1995b) (Fig. 5). These seminomas appeared to contain a mutation in K-RAS or N-RAS. Indeed, the induction of apoptosis of epithelial cells upon disruption of the microenvironment can be inhibited by activated RAS (Arends et al., 1993). A mutated RAS gene in a seminoma does not seem to have prognostic relevance (L. H. J. Looijenga, unpublished). Seminomas with the aforementioned amplification of 12p-sequences (including K-RAS) also show enhanced survival in vitro (H. Roelofs, unpublished). Apparently, the survival of seminoma cells in vitro can be due

Fig. 3. The subsequent steps in the procedure of comparative genomic hybridization are indicated. Control DNA (male) is visualized in red, and tumour DNA (seminoma) is visualized in green, after simultaneous hybridization onto a metaphase spread of a normal male (46XY). The metaphase is stained by G-bandung (a), and 4,6-diamidino-2-phenylindole (DAPI) (b) to allow identification of the individual chromosomes. (c) The hybridization pattern of normal DNA. Note the homogeneous staining along all chromosomes, except in the region of heterochromatin, which is excluded from the analysis. (d) The hybridization pattern of tumour DNA. Note the heterogeneity in intensity along the different chromosomes, for example, the gain of the short arm of chromosome 12. (e) The overlay of the two hybridization patterns, showing the regions of loss and gain of genomic material in the tumour, stained more red and green, respectively.
either to activation of a K-RAS and N-RAS, or to amplification of a specific region of 12p, including K-RAS.

**Oncogenes and tumour suppressor genes.** Studies on the possible role of activation of (proto-)oncogenes in the development of TGCTs are rather limited. RAS genes are rarely mutated in TGCTs (for review, see Olie et al., 1995a). No mutations have been found in the c-KIT gene, the protein of which is consistently found in CIS and seminomas, and in some nonseminoma components (Rajpert-De Meyts and Skakkebaek, 1994; Strohmeyer et al., 1995). Overexpression of c-MYC has been detected in < 10% of nonseminomas (Misaki et al., 1989). In conclusion, the role of (proto-)oncogenes in the development of TGCTs needs further analysis. In this context, the short arm of chromosome 12 is clearly a region of great interest.

Several studies have addressed the loss of heterozygosity in TGCTs, which is indicative of the presence of relevant tumour suppressor genes. However, the data are not consistent, as the short arms of chromosomes 3 and 11 show loss of heterozygosity in a relatively high percentage of cases (Looijenga et al., 1994a; Al-Jehani et al., 1995; Lothe et al., 1995). In addition, in a significant number of TGCTs, deletions have been found on 1p13, 1p22, 1p31.3–p32.2 and 1q32 (Mathew et al., 1994). Monosomy of chromosome 5 was preferentially found in teratomas. The regions 5p15.1–p15.2, 5q11 and 5q34–q35 showed frequent loss of heterozygosity in all other histological types (Murty et al., 1996a). Chromosome 12 showed two regions with loss of heterozygosity: q13 and q22 (Murty et al., 1996b). Of particular interest was the finding of a homozygous deletion of about 2 megabases at 12q22. No candidate genes have been identified in this region so far. Homozygous deletions have also been found on the long arm of chromosome 18, including the DCC (deleted in colorectal cancer) tumour suppressor gene (Murty et al., 1994). The role of DCC in the development of TGCTs has also been investigated in two other studies, in principle supporting earlier results (Peng et al., 1995; Strohmeyer et al., 1997), and it has been suggested that the loss of this gene is related to progression. Studies on the loss of heterozygosity, mutations and expression in TGCTs have failed to establish a significant role in the development of this cancer of the following tumour suppressor genes: NME1 and 2, APC, MCC, RB, WT1, P53, NM23-H1 and H2, and p15. In contrast, it has been suggested that inactivation of p16, mapped to the short arm of chromosome 9, either by mutation or methylation, is involved in the pathogenesis of TGCTs (Chaubert et al., 1997; Heidenreich et al., 1998), although no aberrations within this tumour suppressor gene have yet been reported (Hatta et al., 1995).

**Pathogenetic relationship between seminomas and non-seminomas.** The pathogenetic relationship between seminomas and nonseminomas is still a matter of debate. In fact, two models can be distinguished. One assumes that all histological variants of TGCTs originate independently, while the other assumes that all histological variants of TGCTs originate independently, while the
other assumes the seminoma to be an intermediate stage between CIS and the various nonseminomatous lineages. The latter model is supported by the aforementioned ploidy and the chromosomal analysis of pure seminomas and nonseminomas, the clinical observation that patients with a pure seminoma can develop nonseminomatous metastases (Bredael et al., 1982), and the identification of seminomas with nonseminomatous characteristics, known as ‘intermediate phenotypes’ (Walt et al., 1986; Czaja and Ulbright, 1992; Manivel, 1992). However, the most convincing observation was the finding of common structural chromosomal aberrations, apart from i(12p), in both the seminoma and nonseminoma components of combined tumours (Haddad et al., 1988; Van Echten-Arends et al., 1996).

We hypothesize that the natural, uncomplicated development of TGCTs results in seminomas. In every stage, this process may be complicated by the reprogramming of CIS or seminoma cells to pluripotent embryonal carcinoma cells, the stem cells of nonseminomas. Reprogramming of CIS cells will result in intratubular nonseminomas and eventually in pure nonseminomas, with CIS and the foci of intratubular nonseminomas in the seminiferous tubules. A combined tumour will result when this event takes place after the invasive seminoma has developed. Alternatively, a combined tumour can develop when reprogramming takes place in an invasive seminoma cell. Finally, reprogramming may occur in a metastatic seminoma cell, resulting in pure seminomas with nonseminomatous metastases. The age distribution of seminoma and nonseminoma patients suggests that the chance of reprogramming decreases with age. The capacity for reprogramming is referred to as ‘stem cell potential’ (Skakkebæk et al., 1998). It remains to be shown whether the non-random chromosomal differences between seminomas and nonseminomas are the cause or the effect of the reprogramming. This model explains the observation that the nonseminomas are clinically manifest at an earlier age than the seminomas, and that the age of the patients with a combined tumour is in between, since the event of reprogramming accelerates the clinical manifestation of the tumour.

Early diagnosis. Notwithstanding their low incidence, only about 450 cases per year in the Netherlands, TGCTs are an interesting target for early diagnosis. In males in the age group 15–45 years, it is the most frequent cancer. One in seven deaths in this age group is due to this malignancy. CIS cells can be eradicated using a low dose irradiation, resulting in the prevention of the development of an invasive tumour with minor side effects. High risk groups can be defined: about 5% of the patients with a sporadic TGCT, and 15% of those with a familial form of this cancer, have CIS cells in the contralateral testis. Moreover, it has been suggested that a significant percentage of patients with a TGCT have a genetic predisposition. A possible approach for early diagnosis is detection of an alternative splice variant of the platelet-derived growth factor-α receptor (PDGFα-R). This transcript is not present in normal testicular parenchyma and semen, while it can be detected using a simple PCR method in TGCTs, including parenchyma containing CIS cells (Mosselman et al., 1996). We are currently testing whether this molecular assay can be applied to seminal fluid.

Spermatocytic seminomas

Data on the chromosomal constitution of spermatocytic seminomas are limited. Flow cytometry showed a heterogeneous pattern, with mostly diploid and aneuploid stem lines (for review, see Looijenga et al., 1994). We have karyotyped the first spermatocytic seminoma (Fig. 6). In addition, we applied CGH to four spermatocytic seminomas from three patients (one patient had asynchronous bilateral tumours) (Rosenberg et al., 1997). A totally different pattern of numerical aberrations was found compared with TGCTs and teratomas–yolk sac tumours. The only consistent anomaly was gain of chromosome 9, and most of the aberrations involved whole chromosomes.

Genomic imprinting

The classical Mendelian rules of inheritance do not account for the influence of the parental origin of the alleles. In the early 1980s, it became obvious that this was an oversimplification, at least for the genetics of mammalian cells; the parental origin of the haploid genome does influence the phenotypical outcome (for review, see Looijenga et al., 1998). In fact, the paternal genome is responsible for extra-embryonic differentiation, while the maternal genome mainly supported growth of the soma. This functional difference between the parental genomes was named ‘genomic imprinting’. Subsequent investigations revealed that this process is due to the presence of imprinted genes, that is, genes of which only the paternal or the maternal allele is active under physiological conditions. So far, > 40 genes have been found to be undergoing genomic imprinting, some heterogeneously within species, individuals and tissues. Genomic imprinting is established somewhere during development of the germ cell (Rossant, 1993). Mouse primordial germ cells express both alleles of imprinted genes (Szabo and Mann, 1995), which means that the original pattern of imprinting, present in the mature germ cells, has been erased.

The majority of infantile testis tumours, seminomas and nonseminomas show biallelic expression of imprinted genes (Van Gurp et al., 1994; Verkerk et al., 1997; A. Verkerk, unpublished). These data support the hypothesis that these germ cell tumours originate from an early, erased germ cell. In contrast, spermatocytic seminomas showed different extensiveness of the paternal pattern of genomic imprinting (Verkerk et al., 1997, A. J. M. H. Verkerk, M. C. Dekker, J. W. Oosterhuis and L. H. J. Looijenga, unpublished), indicating that the paternal pattern of genomic imprinting is most likely established at the stage of development of spermatogonia B, from which the spermatocytic seminoma originates.

Animal models

Mouse testicular teratomas are most likely a model for germ cell tumours of the infantile testis: the teratomas and yolk sac tumours. This contention is supported by the demonstration by Walt et al. (1993) that mouse testicular teratomas originate from early germ cells, which switch directly to the phenotype of an embryonal stem cell with the potential of somatic differentiation. This hypothesis is also supported by experiments performed on continuously growing mouse primordial germ cells, which give rise to teratomas upon xenografting (Matsui et al., 1992).
The relationship between the teratomas and yolk sac tumours of the infantile testis is not clear. Prolonged culturing in vivo of embryo-derived teratomas in mice resulted in yolk sac tumours, which were aneuploid (Van Berlo et al., 1990), suggesting that yolk sac tumours may result from tumour progression in teratomas. In this context, the finding that tetraploid cells in mouse chimaeras are found preferentially in the trophodermal lineages is of interest (James et al., 1995). Mechanistic studies on p53 to explain the sensitivity to treatment of germ cell-derived tumours (see above) have been performed on representative cell lines of the mouse teratomas. Therefore, the relevance of p53 for TGCTs has yet to be demonstrated.

On the basis of the absence of nonseminomatous elements, clinical behaviour and age distribution, the canine seminomas are most likely a model for spermatocytic seminomas (Looijenga et al., 1994b). Therefore, it would be of interest to test whether the syntenic region(s) of human chromosome 9 is also gained in these tumours of dogs.

No animal models exist for TGCTs. The identification or generation of such a model would facilitate the testing of different hypotheses on the pathogenesis of this cancer, and this should be one of the main goals of the study of TGCTs in the near future.

**Conclusion**

Epidemiological, clinical, histological, and genetic data support the concept of three distinct entities of germ cell-derived tumours in the human testis: the infantile teratomas–yolk sac tumours, the seminomas and nonseminomas, and the spermatocytic seminomas. Infantile teratomas, benign tumours, originate from diploid germ cells directly switching to an embryonal carcinoma cell prone to somatic differentiation. Malignant progression to a yolk sac tumour is accompanied by aneuploidization. TGCTs originate from a tetraploid germ cell, giving rise to CIS and seminomas, which may undergo reprogramming to nonseminomas. Spermatocytic seminomas, benign tumours, stem from a more differentiated, diploid germ cell, the type B spermatogonium. Spermatocytic seminomas may very rarely progress to sarcomas.

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